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<b>(54) Title:</b> METHODS USING OF FAB I AND COMPOUNDS MODULATING FAB I ACTIVITY  <b>(57) Abstract</b>  Prokaryotic FAB I polypeptides and DNA (RNA) encoding such FAB I and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such FAB I for the treatment of infection, such as bacterial infections. Antagonists against such FAB I and their use as a therapeutic to treat /infections, such as staphylococcal infections are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to the presence of FAB I nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding FAB I and for detecting the polypeptide in a host.		

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## METHODS USING OF FAB I AND COMPOUNDS MODULATING FAB I ACTIVITY

This invention relates, in part, to newly identified polynucleotides and polypeptides; variants and derivatives of these polynucleotides and polypeptides; processes for making these  
5 polynucleotides and these polypeptides, and their variants and derivatives; agonists and antagonists of the polypeptides; and uses of these polynucleotides, polypeptides, variants, derivatives, agonists and antagonists. In particular, in these and in other regards, the invention relates to polynucleotides and polypeptides of staphylococcal Fab I enoyl-ACP reductase, hereinafter referred to as "FAB I".

### 10 BACKGROUND OF THE INVENTION

Although the overall pathway of saturated fatty acid biosynthesis is similar in all organisms, the fatty acid synthase (FAS) systems vary considerably with respect to their structural organization. Thus in Type I FAS systems, found in vertebrates and yeasts, the necessary enzymes required for fatty acid synthesis are present on one or two polypeptide  
15 chains respectively. In contrast, in Type II systems found in most bacteria and plants, each step in the pathway is catalysed by a separate mono-functional enzyme. It would therefore appear that significant selectivity of inhibition of the bacterial and mammalian enzymes is possible.

Fab I (previously designated EnvM) functions as an enoyl-acyl carrier protein  
20 (ACP) reductase (Bergler, et al, (1994), *J.Biol.Chem.* 269, 5493-5496) in the final step of the four reactions involved in each cycle of bacterial fatty acid biosynthesis.

The first step is catalysed by  $\beta$ -ketoacyl-ACP synthase, which condenses malonyl-ACP with acetyl-CoA (FabH, synthase III). In subsequent rounds malonyl-ACP is condensed with the growing-chain acyl-ACP (FabB and FabF, synthases I and II  
25 respectively).

The second step in the elongation cycle is ketoester reduction by NADPH-dependent  $\beta$ -ketoacyl-ACP reductase (FabG). Subsequent dehydration by  $\beta$ -hydroxyacyl-ACP dehydrase (either FabA or FabZ) leads to trans-2-enoyl-ACP which is in turn converted to acyl-ACP by NADH-dependent enoyl-ACP reductase (Fab I). Further rounds  
30 of this cycle, adding two carbon atoms per cycle, eventually lead to palmitoyl-ACP (16C) where upon the cycle is stopped largely due to feedback inhibition of Fab I by palmitoyl-

ACP (Heath, et al, (1996), *J.Biol.Chem.* 271, 1833-1836). Fab I is therefore a major biosynthetic enzyme which is also a key regulatory point in the overall synthetic pathway.

Early data suggested that there were two enoyl-ACP reductases in *E.coli*, one NADPH dependent and the other NADH dependent. However, more recent work has found  
5 no evidence for the NADPH dependent enzyme and Fab I is the only enoyl ACP reductase identified in *E.coli*. (Heath, et al, (1995), *J.Biol.Chem.* 270, 26538 - 26542; Bergler, et al, (1994), *J.Biol.Chem.* 269, 5493-5496).

It has been shown that diazaborine antibiotics inhibit fatty acid, phospholipid and lipopolysaccharide (LPS) biosynthesis and it has also been shown that the antibacterial  
10 target of these compounds is Fab I. For example derivative 2b18 from Grassberger, et al (1984) *J. Med Chem* 27 947-953 has been shown to be a non-competitive inhibitor of Fab I having a  $K_i = 0.2\text{mM}$  (Bergler, et al, (1994), *J.Biol.Chem.* 269, 5493-5496). The antibacterial activity of diazaborine derivatives against Gram-negatives and Gram positive organisms is well documented (Grassberger et al., *J Med Chem.* 1984 27, 947-953;  
15 Gronowitz et al., *Acta Pharm Suecica*, 1971 8 377; Wersch et al U.S. Patent, 2,533,918; Lam et al., *J. Antimicrob. Chemother.* 1987 20 37-45).

Conditionally lethal Fab I mutants have been constructed in *E.coli* and the *Fab I* gene from *Salmonella typhimurium* complements this mutation. In addition, plasmids containing the *Fab I* gene from diazaborine resistant *S. typhimurium* conferred diazaborine  
20 resistance in *E.coli* (Turnowsky, et al, (1989), *J.Bacteriol.*, 171, 6555-6565) confirming Fab I as the antibacterial target of diazaborines.

Inhibition of Fab I either by diazaborine or by raising the temperature in an Fab I temperature sensitive mutant to non-permissive conditions is lethal, thus demonstrating that Fab I is essential to the survival of the organism (Bergler, et al, (1994), *J.Biol.Chem.* 269,  
25 5493-5496). Laboratory generated point mutations in the *Fab I* gene lead to diazaborine resistant *E.coli*.

Fab I is conserved in Gram negative organisms with 98% identity between *E.coli* and *S.typhimurium* Fab I (Bergler, et al, (1992), *J.Gen.Microbiol.* 138. 2093-2100) and 75% identity between these proteins and *H.influenzae* Fab I. *Staphylococcus aureus* FAB I of the invention shows 54% similarity to the mycobacterial protein, InhA, which is highly conserved throughout mycobacteria including *M.tuberculosis*. *E.coli* Fab I was found to be 34% identical, 57% similar to *Brassica napus* (rape seed) enoyl-ACP reductase and *S. aureus* FAB I of the present invention was also 34% identical, 57% similar. Moreover, FAB I of the present invention was found to be 44% identical, 64% similar over 252 amino acids to *E.coli* Fab I. FAB I of the present invention is only 27% identical, 48% similar to a mammalian 2,4-dienoyl-coenzyme A reductase. This mammalian homolog differs from FAB I in that it is involved in the  $\beta$ -oxidation of polyunsaturated enoyl-CoAs and utilizes NADPH as cofactor rather than NADH. Therefore, there is significant potential for selective inhibition of FAB I. Since there are no marketed antibiotics targeted against fatty acid biosynthesis it is likely that inhibitors of FAB I will not be susceptible to current antibiotic resistance mechanisms. Moreover, this is a potentially broad spectrum target.

There is an unmet need for developing new classes of antibiotic compounds. Clearly, there is also a need for factors, such as FAB I, that may be used to screen compounds for antibiotic activity, such as a simple high through-put assay for screening inhibitors of FAS. Such factors may also be used to determine their roles in pathogenesis of infection, dysfunction and disease. Identification and characterization of such factors, which can play a role in preventing, ameliorating or correcting infections, dysfunctions or diseases are critical steps in making important discoveries to improve human health.

#### SUMMARY OF THE INVENTION

It is an object of the present invention, there are provided FAB I agonists. Among preferred agonists are molecules that mimic FAB I, that bind to FAB I-binding molecules or binding molecules, and that elicit or augment FAB I-induced responses. Also among preferred agonists are molecules that interact with FAB I, or with other modulators of FAB I activities, and thereby potentiate or augment an effect of FAB I or more than one effect of FAB I and are bacteriostatic or bacteriocidal.

In accordance with yet another aspect of the present invention, there are provided FAB I antagonists. Among preferred antagonists are those which mimic FAB I so as to bind to FAB I-binding molecules but not elicit a FAB I-induced response or more than one FAB I-

induced response. Also among preferred antagonists are molecules that bind to or interact with FAB I so as to inhibit an effect of FAB I or more than one effect of FAB I or which prevent expression of FAB I. Further particularly preferred antagonists of FAB I lower or abolish a FabI enzymatic activity or activities.

- 5           In a further aspect of the invention there are provided compositions comprising a FAB I polynucleotide or a FAB I polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a FAB I polynucleotide for expression of a FAB I polypeptide in a host organism to raise an immunological response, preferably to raise immunity in such host against bacteria, preferably staphylococci or closely genetically related organisms.

The following further embodiments are also provided by the invention:

- 15           An antagonist that inhibits or an agonist that activates an activity a polypeptide selected from the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, wherein said activity is selected from the group consisting of:
- 20           uncompetitive inhibition by Apo-ACP versus NADH (K<sub>i</sub>(app);  
competitive inhibition by Apo-ACP versus crotonoyl CoA;  
induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
binding of NADH and NADPH by FabI;  
oxidation of NADH and NADPH by FabI;  
ratio of K<sub>m</sub>app for NADH as compared to NADPH;
- 25           use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;  
sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty
- 30           acyl CoA's;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA;

- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation
- through binding of multiple palmitoyl CoA molecules to Fab I;
- binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;
- 5 formation of an dimeric quaternary structure;
- formation of an tetrameric quaternary structure;
- formation of an oligomeric quaternary structure;
- binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl
- coA; and
- 10 NADH binding to Fab I prior to or simultaneous with ACP binding.

A method for the treatment of an individual having need to inhibit or activate Fab I polypeptide comprising the steps of: administering to the individual a antibacterially effective amount of an antagonist that inhibits or an agonist that

15 activates an activity of a polypeptide selected from the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, wherein said activity is selected from the group consisting of:

- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );
- 20 competitive inhibition by Apo-ACP versus crotonoyl CoA;
- induction of negative cooperativity with respect to CCA binding;
- use of NADH and NADPH as substrates by Fab I;
- binding of NADH and NADPH by FabI;
- oxidation of NADH and NADPH by FabI;
- 25 ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;
- use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
- sequential binding of NADH and crotonoyl CoA by Fab I;
- increasing inhibition of FabI by saturated fatty acyl CoA's of increasing
- 30 chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA;

- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation
- through binding of multiple palmitoyl CoA molecules to Fab I;
- binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;
- 5 formation of an dimeric quaternary structure;
- formation of an tetrameric quaternary structure;
- formation of an oligomeric quaternary structure;
- binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl
- coA; and
- 10 NADH binding to Fab I prior to or simultaneous with ACP binding.

- A method for the treatment of an individual infected with a bacteria comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits or an agonist that activates an activity of a polypeptide selected from the group consisting of: a polypeptide comprising an amino
- 15 acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, wherein said activity is selected from the group consisting of:
  - uncompetitive inhibition by Apo-ACP versus NADH ( $K_{i(app)}$ );
  - competitive inhibition by Apo-ACP versus crotonoyl CoA;
  - 20 induction of negative cooperativity with respect to CCA binding;
  - use of NADH and NADPH as substrates by Fab I;
  - binding of NADH and NADPH by FabI;
  - oxidation of NADH and NADPH by FabI;
  - ratio of  $K_{mapp}$  for NADH as compared to NADPH;
  - 25 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
  - sequential binding of NADH and crotonoyl CoA by Fab I;
  - increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty
  - 30 acyl CoA's;
  - competitive inhibition by palmitoyl CoA versus crotonoyl CoA;



- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
- binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;
- 5 formation of an dimeric quaternary structure;
- formation of an tetrameric quaternary structure;
- formation of an oligomeric quaternary structure;
- binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl coA; and
- 10 NADH binding to Fab I prior to or simultaneous with ACP binding.

A method for the treatment of an individual having need to inhibit or activate Fab I polypeptide comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits or an agonist that activates an activity of Fab I selected from the group consisting of:

- 15 uncompetitive inhibition by Apo-ACP versus NADH (Ki(app);
- competitive inhibition by Apo-ACP versus crotonoyl CoA;
- induction of negative cooperativity with respect to CCA binding;
- use of NADH and NADPH as substrates by Fab I;
- binding of NADH and NADPH by FabI;
- 20 oxidation of NADH and NADPH by FabI;
- ratio of Kmapp for NADH as compared to NADPH;
- use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
- sequential binding of NADH and crotonoyl CoA by Fab I;
- 25 increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
- 30 binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;

formation of an dimeric quaternary structure;  
formation of an tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
5 coA; and  
NADH binding to Fab I prior to or simultaneous with ACP binding.

A method for the treatment of an individual infected with a bacteria comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits or an agonist that activates that activates an  
10 activity of Fab I selected from the group consisting of:

uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );  
competitive inhibition by Apo-ACP versus crotonoyl CoA;  
induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
15 binding of NADH and NADPH by FabI;  
oxidation of NADH and NADPH by FabI;  
ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;  
use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;  
20 sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;

competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
25 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
negative cooperativity in the binding of CCA;  
formation of an dimeric quaternary structure;  
30 formation of an tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;

binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
coA; and

NADH binding to Fab I prior to or simultaneous with ACP binding.

- 5 A method for the treatment of an individual infected by *Streptococcus pneumoniae* comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits or anagonist that activates an activity of *Streptococcus pneumoniae* Fab I selected from the group consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH (Ki(app);  
competitive inhibition by Apo-ACP versus crotonoyl CoA;  
10 induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
binding of NADH and NADPH by FabI;  
oxidation of NADH and NADPH by FabI;  
ratio of Kmapp for NADH as compared to NADPH;  
15 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;  
sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty  
20 acyl CoA's;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
25 negative cooperativity in the binding of CCA;  
formation of an dimeric quaternary structure;  
formation of an tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
30 coA; and  
NADH binding to Fab I prior to or simultaneous with ACP binding.

- An antagonist that inhibits an activity of a polypeptide selected from the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, .
- 5 wherein said activity is selected from the group consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );
  - competitive inhibition by Apo-ACP versus crotonoyl CoA;
  - induction of negative cooperativity with respect to CCA binding;
  - use of NADH and NADPH as substrates by Fab I;
  - 10 binding of NADH and NADPH by FabI;
  - oxidation of NADH and NADPH by FabI;
  - ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;
  - use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
  - 15 sequential binding of NADH and crotonoyl CoA by Fab I;
  - increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
  - competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
  - 20 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
  - binding of multiple palmitoyl CoA molecules to Fab I;
  - negative cooperativity in the binding of CCA;
  - formation of an dimeric quaternary structure;
  - 25 formation of an tetrameric quaternary structure;
  - formation of an oligomeric quaternary structure;
  - binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl coA; and
  - NADH binding to Fab I prior to or simultaneous with ACP binding.
  - 30 A method for the treatment of an individual having need to inhibit Fab I polypeptide comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits an activity of a polypeptide selected

from the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, wherein said activity is selected from the group consisting of:

- 5 uncompetitive inhibition by Apo-ACP versus NADH ( $K_{iapp}$ );
- competitive inhibition by Apo-ACP versus crotonoyl CoA;
- induction of negative cooperativity with respect to CCA binding;
- use of NADH and NADPH as substrates by Fab I;
- binding of NADH and NADPH by FabI;
- 10 oxidation of NADH and NADPH by FabI;
- ratio of  $K_{mapp}$  for NADH as compared to NADPH;
- use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
- sequential binding of NADH and crotonoyl CoA by Fab I;
- 15 increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation
- 20 through binding of multiple palmitoyl CoA molecules to Fab I;
- binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;
- formation of an dimeric quaternary structure;
- formation of an tetrameric quaternary structure;
- 25 formation of an oligomeric quaternary structure;
- binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl coA; and
- NADH binding to Fab I prior to or simultaneous with ACP binding.

30 A method for inhibiting an activity of Fab I polypeptide comprising the steps of contacting a composition comprising said polypeptide with an effective amount of an antagonist that inhibits an activity of Fab I, wherein said activity is selected from the group consisting of:

- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );
- competitive inhibition by Apo-ACP versus crotonoyl CoA;
- induction of negative cooperativity with respect to CCA binding;
- use of NADH and NADPH as substrates by Fab I;
- 5 binding of NADH and NADPH by FabI;
- oxidation of NADH and NADPH by FabI;
- ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;
- use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
- 10 sequential binding of NADH and crotonoyl CoA by Fab I;
- increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
- 15 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
- binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;
- formation of an dimeric quaternary structure;
- 20 formation of an tetrameric quaternary structure;
- formation of an oligomeric quaternary structure;
- binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl coA; and
- NADH binding to Fab I prior to or simultaneous with ACP binding.
- 25 A method for inhibiting an activity of Fab I, wherein said activity is selected from the group consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );
- competitive inhibition by Apo-ACP versus crotonoyl CoA;
- induction of negative cooperativity with respect to CCA binding;
- 30 use of NADH and NADPH as substrates by Fab I;
- binding of NADH and NADPH by FabI;
- oxidation of NADH and NADPH by FabI;

ratio of  $K_{mapp}$  for NADH as compared to NADPH;  
 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
 kinetic mechanism;  
 sequential binding of NADH and crotonoyl CoA by Fab I;  
 5 increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
 chain length; feedback regulatory mechanism of Fab I by saturated fatty  
 acyl CoA's;  
 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
 10 through binding of multiple palmitoyl CoA molecules to Fab I;  
 binding of multiple palmitoyl CoA molecules to Fab I;  
 negative cooperativity in the binding of CCA;  
 formation of an dimeric quaternary structure;  
 formation of an tetrameric quaternary structure;  
 15 formation of an oligomeric quaternary structure;  
 binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl  
 coA; and  
 NADH binding to Fab I prior to or simultaneous with ACP binding.

A method for inhibiting a growth of bacteria comprising the steps of  
 20 contacting a composition comprising bacteria with an antibacterially effective amount  
 of an antagonist that inhibits an activity of Fab I, wherein said activity is selected  
 from the group consisting of:  
 uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(ACP)$ );  
 competitive inhibition by Apo-ACP versus crotonoyl CoA;  
 25 induction of negative cooperativity with respect to CCA binding;  
 use of NADH and NADPH as substrates by Fab I;  
 binding of NADH and NADPH by FabI;  
 oxidation of NADH and NADPH by FabI;  
 ratio of  $K_{mapp}$  for NADH as compared to NADPH;  
 30 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
 kinetic mechanism;  
 sequential binding of NADH and crotonoyl CoA by Fab I;

- increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
- 5 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
- binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;
- formation of an dimeric quaternary structure;
- 10 formation of an tetrameric quaternary structure;
- formation of an oligomeric quaternary structure;
- binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl coA; and
- NADH binding to Fab I prior to or simultaneous with ACP binding.
- 15 A method for inhibiting a Fab I polypeptide comprising the steps of contacting a composition comprising bacteria with an antibacterially effective amount of an antagonist that inhibits an activity of Fab I, wherein said activity is selected from the group consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );
- 20 competitive inhibition by Apo-ACP versus crotonoyl CoA;
- induction of negative cooperativity with respect to CCA binding;
- use of NADH and NADPH as substrates by Fab I;
- binding of NADH and NADPH by FabI;
- oxidation of NADH and NADPH by FabI;
- 25 ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;
- use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
- sequential binding of NADH and crotonoyl CoA by Fab I;
- increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty
- 30 acyl CoA's;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA;



- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
 through binding of multiple palmitoyl CoA molecules to Fab I;  
 binding of multiple palmitoyl CoA molecules to Fab I;  
 negative cooperativity in the binding of CCA;  
 5 formation of an dimeric quaternary structure;  
 formation of an tetrameric quaternary structure;  
 formation of an oligomeric quaternary structure;  
 binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
 coA; and  
 10 NADH binding to Fab I prior to or simultaneous with ACP binding.

It is preferred in the methods of the invention that any bacteria is selected from the group consisting of: a member of the genus *Staphylococcus*, *Staphylococcus aureus*, a member of the genus *Streptococcus*, and *Streptococcus pneumoniae*.

- Other objects, features, advantages and aspects of the present invention will  
 15 become apparent to those of skill from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the  
 20 other parts of the present disclosure.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The following drawing depicts certain embodiments of the invention. It is illustrative only and does not limit the invention otherwise disclosed herein.

- Figure 1 shows the cycle of fatty acid elongation in bacteria  
 25 Figure 2 shows a chemical synthesis of deuterated pyridine nucleotides  
 Figure 3 shows predicted and observed product structures and (M+H)<sup>+</sup>s based on *E. coli* Fab I.  
 Figure 4 (A, B, and C) shows mass spectra of components from reaction 3 of Figure  
 3.  
 30 Figure 5 shows a stereochemical course of *S. aureus* Fab I  
 Figure 6 shows graphically that *S. aureus* Fab I uses both NADPH and NADH as substrates.

Figure 7 shows graphically that *S. aureus* Fab I exhibits a sequential reaction mechanism.

Figure 8 shows a pH profile of *S. aureus* Fab I

Figure 9 shows inhibition by palmitoyl CoA

5 Figure 10 shows inhibition by beta-NADP<sup>+</sup>

Figure 11 shows a kinetic model for inhibition by beta-NADP<sup>+</sup>

Figure 12 shows graphically that inhibition by apo-ACP vs. NADH

Figure 13 shows graphically the inhibition by apo-ACP vs. CCA

Figure 14 shows minimal kinetic mechanism for inhibition by apo-ACP

10 Figure 15 shows induced cooperative inhibition by apo-ACP

Figure 16 [SEQ ID NO:2] shows the polypeptide of *Staphylococcus aureus* FAB I deduced from the polynucleotide of Figure 17 [SEQ ID NO:1].

Figure 17 [SEQ ID NO:1] shows the polynucleotide sequence of *Staphylococcus aureus* FAB I.

## 15 GLOSSARY

The following illustrative explanations are provided to facilitate understanding of certain terms used frequently herein, particularly in the Examples. The explanations are provided as a convenience and are not limitative of the invention.

20 "Binding molecule(s)," as used herein, refers to molecules or ions which bind or interact specifically with FAB I polypeptides or polynucleotides of the present invention, including, for example enzyme substrates and substrate and co-factor mimetics, as well as classical receptors (which also may be referred to as "binding molecules" and "interaction molecules," respectively and as "FAB I binding molecules" and "FAB I interaction molecules"). Binding between polypeptides of the invention and such molecules, including

25 binding or binding or interaction molecules may be exclusive to polypeptides of the invention, which is very highly preferred, or it may be highly specific for polypeptides of the invention, which is highly preferred, or it may be highly specific to a group of proteins that includes polypeptides of the invention, which is preferred, or it may be specific to several groups of proteins at least one of which includes a polypeptide of the invention.

30 Binding molecules also may be non-naturally occurring, such as antibodies and antibody-derived reagents that bind specifically to polypeptides of the invention.

"Digestion of DNA" refers to cleavage of DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes referred to herein are

commercially available and their reaction conditions, cofactors and other requirements for use are well known and routine to the skilled artisan.

For analytical purposes, typically, 1  $\mu$ g of plasmid or DNA fragment is digested with about 2 units of enzyme in about 20  $\mu$ l of reaction buffer. For the purpose of isolating DNA  
 5 fragments for plasmid construction, typically 5 to 50  $\mu$ g of DNA are digested with 20 to 250 units of enzyme in proportionately larger volumes.

Appropriate buffers and substrate amounts for particular restriction enzymes are described in standard laboratory manuals, such as those referenced below, and they are specified by commercial suppliers.

10 Incubation times of about 1 hour at 37°C are ordinarily used, but conditions may vary in accordance with standard procedures, the supplier's instructions and the particulars of the reaction. After digestion, reactions may be analyzed, and fragments may be purified by electrophoresis through an agarose or polyacrylamide gel, using well known methods that are routine for those skilled in the art.

15 "Disease(s)" means (i) bacterial infections, such as staphylococcal infections including, but not limited to infections of the upper respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), the lower respiratory tract (e.g., empyema, lung abscess), the cardiac system (e.g., infective endocarditis), the gastrointestinal tract (e.g., secretory diarrhoea, splenic abscess, retroperitoneal abscess), the CNS (e.g., cerebral abscess),  
 20 eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), the kidney or urinary tract (e.g., epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), the skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis), and the bones and joints (e.g. septic arthritis, osteomyelitis) and/or (ii) an infection caused by or related to a member of the genus  
 25 *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*, *Enterobacter*, *Yersinia*, *Fancisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*, *Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Klebsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*,  
 30 *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not limited to, a member of the species or group, Group A *Streptococcus*, Group B *Streptococcus*, Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus*

*pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*,  
*Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*,  
*Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella vaginalis*,  
*Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*, *Mycobacterium*  
5 *leprae*, *Actinomyces israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella*  
*parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus*  
*influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*,  
*Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*,  
*Yersinia pestis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio*  
10 *cholera*, *Shigella dysenterii*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Fransciscella*  
*tularensis*, *Brucella abortis*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*,  
*Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii* and  
*Chlamydia trachomatis*.

"Genetic Element(s)" means a polynucleotide comprising a region that encodes a  
15 polypeptide or a region that regulates transcription or translation or other processes important  
to expression of the polypeptide in a host cell, or a polynucleotide comprising both a region  
that encodes a polypeptide and a region operably linked thereto that regulates expression.

Genetic elements may be comprised within a vector that replicates as an episomal  
element; that is, as a molecule physically independent of the host cell genome. They may be  
20 comprised within plasmids. Genetic elements also may be comprised within a host cell  
genome; not in their natural state but, rather, following manipulation such as isolation, cloning  
and introduction into a host cell in the form of purified DNA or in a vector, among others.

"Host cell(s)" means a cell which has been transformed, transfected, infected, or  
entered by an exogenous polynucleotide sequence, or is capable of transformation,  
25 infection, transfection, or entry by an exogenous polynucleotide sequence.

"Identity", as known in the art, means a relationships between two polypeptide  
sequences or two polynucleotide sequences, as determined by comparing the sequences. In  
the art, identity also means the degree of sequence relatedness between two polypeptide or  
two polynucleotide sequences as determined by the match between two strings of such  
30 sequences. Both identity and similarity can be readily calculated (*Computational*  
*Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988;  
*Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New

York, 1993; *Computer Analysis of Sequence Data*, Part I. Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of  
5 methods to measure identity and similarity between two polynucleotide or two polypeptide sequences, both terms are well known to skilled artisans (*Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math.*, 48: 1073 (1988). Methods commonly employed to determine  
10 identity or similarity between two sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM *J. Applied Math.*, 48:1073 (1988). Preferred methods to determine identity are designed to give the largest match between the two sequences tested. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity  
15 between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., (1984) *Nucleic Acids Research* 12(1): 387), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., (1990) *J. Molec. Biol.* 215: 403).

"Isolated" means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For  
20 example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism,  
25 which organism may be living or non-living.

As part of or following isolation, such polynucleotides can be joined to other polynucleotides, such as DNAs, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated polynucleotides, alone or joined to other polynucleotides such as vectors, can be introduced into host cells, in culture or in whole  
30 organisms. Introduced into host cells in culture or in whole organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the polynucleotides and polypeptides may occur in a composition, such as a media formulations, solutions for introduction of polynucleotides or

polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated polynucleotides or polypeptides within the meaning of that term as it is employed herein.

5 "Ligation" means a process of forming phosphodiester bonds between two or more polynucleotides, which most often are double stranded DNAs. Techniques for ligation are well known to the art and protocols for ligation are described in standard laboratory manuals and references, such as, for instance, Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring  
10 Harbor, New York (1989) and Maniatis et al., pg. 146, as cited below.

"Oligonucleotide(s)" refers to relatively short polynucleotides. Often the term refers to single-stranded deoxyribonucleotides, but it can refer as well to single-or double-stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs, among others.

Oligonucleotides, such as single-stranded DNA probe oligonucleotides, often are  
15 synthesized by chemical methods, such as those implemented on automated oligonucleotide synthesizers. However, oligonucleotides can be made by a variety of other methods, including *in vitro* recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms.

Initially, chemically synthesized DNAs typically are obtained without a 5' phosphate.  
20 The 5' ends of such oligonucleotides are not substrates for phosphodiester bond formation by ligation reactions that employ DNA ligases typically used to form recombinant DNA molecules. Where ligation of such oligonucleotides is desired, a phosphate can be added by standard techniques, such as those that employ a kinase and ATP.

The 3' end of a chemically synthesized oligonucleotide generally has a free hydroxyl  
25 group and, in the presence of a ligase, such as T4 DNA ligase, readily will form a phosphodiester bond with a 5' phosphate of another polynucleotide, such as another oligonucleotide. As is well known, this reaction can be prevented selectively, where desired, by removing the 5' phosphates of the other polynucleotide(s) prior to ligation.

"Organism(s)" when used herein with reference to a life form used for cloning or that  
30 causes (or is believed to cause) disease means (i) a prokaryote, including but not limited to, a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*, *Haemophilus*, *Actinomycetes*, *Streptomyces*, *Nocardia*, *Enterobacter*, *Yersinia*, *Fancisella*, *Pasturella*, *Moraxella*, *Acinetobacter*, *Erysipelothrix*,

*Branhamella*, *Actinobacillus*, *Streptobacillus*, *Listeria*, *Calymmatobacterium*, *Brucella*, *Bacillus*, *Clostridium*, *Treponema*, *Escherichia*, *Salmonella*, *Klebsiella*, *Vibrio*, *Proteus*, *Erwinia*, *Borrelia*, *Leptospira*, *Spirillum*, *Campylobacter*, *Shigella*, *Legionella*, *Pseudomonas*, *Aeromonas*, *Rickettsia*, *Chlamydia*, *Borrelia* and *Mycoplasma*, and further including, but not  
5 limited to, a member of the species or group, Group A *Streptococcus*, Group B *Streptococcus*, Group C *Streptococcus*, Group D *Streptococcus*, Group G *Streptococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus durans*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae*, *Gardnerella*  
10 *vaginalis*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium ulcerans*, *Mycobacterium leprae*, *Actinomyces israelii*, *Listeria monocytogenes*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Escherichia coli*, *Shigella dysenteriae*, *Haemophilus influenzae*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus ducreyi*, *Bordetella*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*,  
15 *Yersinia pestis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Vibrio cholera*, *Shigella dysenteriae*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Francisella tularensis*, *Brucella abortus*, *Bacillus anthracis*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*, *Treponema pallidum*, *Rickettsia rickettsii* and *Chlamydia trachomatis*, (ii) an archaeon, including but not limited to *Archaeobacter*.

20 "Plasmid(s)" generally are designated herein by a lower case p preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art.

Starting plasmids disclosed herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids by routine  
25 application of well known, published procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art. Moreover, those of skill readily may construct any number of other plasmids suitable for use in the invention. The properties, construction and use of such plasmids, as well as other vectors, in the present invention will be readily  
30 apparent to those of skill from the present disclosure.

"Polynucleotide(s)" means any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance,

polynucleotides as used herein refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-  
5 stranded or, more typically, double-stranded, or triple-stranded, or a mixture of single- and double-stranded regions.

In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the  
10 molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide.

As used herein, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover,  
15 DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein.

It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or  
20 metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, *inter alia*.

"Polypeptide(s)", as used herein, includes all polypeptides as described below. The basic structure of polypeptides is well known and has been described in innumerable textbooks and other publications in the art. In this context, the term is used herein to refer to  
25 any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types.

30 It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids, and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes, such as processing and other post-translational modifications, but



also by chemical modification techniques which are well known to the art. Even the common modifications that occur naturally in polypeptides are too numerous to list exhaustively here, but they are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art.

5           Among the known modifications which may be present in polypeptides of the present are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, 10 demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

15           Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. 20 H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., *Posttranslational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., (1990) *Meth. Enzymol.* 182:626-646 and Rattan et al., (1992) *Protein Synthesis: 25 Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663: 48-62.

          It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human 30 manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural process and by entirely synthetic methods, as well.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli* or other cells, prior to proteolytic processing, almost invariably will be N-formylmethionine. During post-translational modification of the peptide, a methionine residue at the NH<sub>2</sub>-terminus may be deleted. Accordingly, this invention contemplates the use of both the methionine-containing and the methionineless amino terminal variants of the protein of the invention.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell posttranslational modification capacity and the modification signals present in the polypeptide amino acid sequence. For instance, as is well known, glycosylation often does not occur in bacterial hosts such as, for example, *E. coli*. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell.

It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

"Transformation" means the process by which a cell is "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is often one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

"Variant(s)" of polynucleotides or polypeptides, as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide, respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail.

- 5 (1) A polynucleotide that differs in nucleotide sequence from another, reference polynucleotide. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical.

As noted below, changes in the nucleotide sequence of the variant may be silent. That is, they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference. Also as noted below, changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below.

10  
15

- (2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many region, identical.

A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

20

#### DESCRIPTION OF THE INVENTION

Fab I functions as the final step of the elongation cycle of bacterial fatty acid biosynthesis. The enzyme from *S. aureus*, has been cloned, overexpressed in *E. coli* and purified to >95% purity. Kinetic studies revealed sequential addition of the substrates crotonoyl CoA and NADH over the broad optimal pH range of about or equal to 5.0-7.0. The lack of a demonstrable back reaction or product inhibition prevented discrimination between an ordered versus a random mechanism. The  $K_m$  for crotonoyl CoA was virtually identical to that of the *E. coli* Fab I (about or equal to 2.5 mM), but the  $K_m$  for NADH was approximately 30-fold higher (about or equal to 410  $\mu$ M versus 13  $\mu$ M for *E. coli*). Studies using deuterated substrates revealed a stereochemical course of the Fab I reaction identical to that reported for the *E. coli* enzyme: the hydride is transferred from the B-face of the nicotinamide ring of NADH, and reduction occurs by a *syn* addition of hydrogen at the pro-2R and pro-3S positions of the double bond of crotonoyl CoA. Inhibition of *S. aureus* Fab I

25  
30

by a series of eight fatty acyl CoA's from acetyl to palmitoyl increases with the chain length of the acyl group, suggesting that, like *E. coli* Fab I, *S. aureus* Fab I is subject to feedback inhibition by long chain acyl-ACP's. This characteristic presumably plays a key regulatory role *in vivo*.

5       The present invention relates to novel FAB I polypeptides and polynucleotides, among other things, as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of a novel FAB I gene of *Staphylococcus aureus*, which is related by amino acid sequence homology to Fab I enzymes from Mycobacteria (InhA), *H.influenzae*, *Brassica napus* (rape seed) enoyl-ACP reductase, and to the *E.coli* Fab I  
10       protein.

      The invention relates especially to FAB I having the nucleotide and amino acid sequences set out in Figure 17 [SEQ ID NO:1] and Figure 16 [SEQ ID NO:2] respectively, and to the FAB I nucleotide sequences in NCIMB Deposit No. 40771 and amino acid sequences encoded therefrom, which is herein referred to as "the deposited clone" or as the  
15       "DNA of the deposited clone." It will be appreciated that the nucleotide and amino acid sequences set out in Figure 16 [SEQ ID NO:2] and Figure 17 [SEQ ID NO:1] were obtained by sequencing the FAB I DNA of the deposited clone. Hence, the sequence of the deposited clone is controlling as to any discrepancies between it (and the sequence it encodes) and the sequences of Figure 16 [SEQ ID NO:2] or Figure 17 [SEQ ID NO:1].

## 20       **Polynucleotides**

      In accordance with one aspect of the present invention, there are provided isolated polynucleotides which encode the FAB I polypeptide having the deduced amino acid sequence of figure 16 [SEQ ID NO:2].

      Using the information provided herein, such as the polynucleotide sequence set out  
25       in figure 17 [SEQ ID NO:1], a polynucleotide of the present invention encoding FAB I polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning genomic DNAs from bacteria using *Staphylococcus aureus* WCUH29 cells as starting material. Illustrative of the invention, the polynucleotide set out in figure 17 [SEQ ID NO:1] was discovered in a genomic DNA library derived from *Staphylococcus aureus*  
30       WCUH29.

      FAB I of the invention is structurally related to other proteins of the enoyl-ACP reductase family, as shown by the results of sequencing the genomic DNA encoding FAB I in the deposited clone. The DNA sequence thus obtained is set out in figure 17 [SEQ ID NO:1].

It contains an open reading frame encoding a protein of about 256 amino acid residues with a deduced molecular weight of about 27.99 kDa. The protein exhibits greatest homology to the *E. coli* Fab I protein among known proteins. FAB I offfigure 16 [SEQ ID NO:2] has about 44% identity and about 64% similarity with the amino acid sequence of *E. coli* enoyl (ACP) reductase (Fab I), SwissProt Accession Number P29132.

Polynucleotides of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The coding sequence which encodes the polypeptide may be identical to the coding sequence of the polynucleotide shown in figure 17 [SEQ ID NO:1]. It also may be a polynucleotide with a different sequence, which, as a result of the redundancy (degeneracy) of the genetic code, encodes the polypeptide of the DNA offfigure 16 [SEQ ID NO:2].

Polynucleotides of the present invention which encode the polypeptide offfigure 16 [SEQ ID NO:2] may include, but are not limited to the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription (including termination signals, for example), ribosome binding, mRNA stability elements, and additional coding sequence which encode additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain embodiments of this aspect of the invention, the marker sequence is a hexa-histidine as discussed, this should include other tagging approaches peptide, such as the tag provided in the pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al., (1989) *Proc. Natl. Acad. Sci., USA* 86: 821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag may also be used to create fusion

proteins and corresponds to an epitope derived of influenza hemagglutinin protein, which has been described by Wilson et al., (1984) *Cell* 37: 767, for instance.

In accordance with the foregoing, the term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides which include a sequence encoding a polypeptide of the present invention, particularly staphylococcal FAB I having the amino acid sequence set out in figure 16 [SEQ ID NO:2]. The term encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage, insertion sequence, recombination, or editing) together with additional regions, that also may contain coding and/or non-coding sequences.

The present invention further relates to variants of the herein above described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of figure 16 [SEQ ID NO:2]. A variant of the polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Among the particularly preferred embodiments of the invention in this regard are polynucleotides encoding polypeptides having the amino acid sequence of FAB I set out in figure 16 [SEQ ID NO:2]; variants, analogs, derivatives and fragments thereof, and fragments of the variants, analogs and derivatives.

Further particularly preferred in this regard are polynucleotides encoding FAB I variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence of FAB I polypeptide of figure 16 [SEQ ID NO:2] in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of FAB I. Also especially preferred in this regard are conservative substitutions. Most highly preferred

are polynucleotides encoding polypeptides having the amino acid sequence of figure 16 [SEQ ID NO:2], without substitutions.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical to a polynucleotide encoding FAB I polypeptide having the amino acid sequence set out in figure 16 [SEQ ID NO:2], and polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical to a polynucleotide encoding FAB I polypeptide encoded by the *Staphylococcus aureus* DNA of the deposited clone and polynucleotides complementary thereto or as set out in figure 17 [SEQ ID NO:1]. In this regard, polynucleotides at least 90% identical to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Particularly preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the DNA of figure 17 [SEQ ID NO:1].

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding FAB I and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the FAB I gene. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly preferred probes will have at least 30 bases and will have 50 bases or less.

For example, the coding region of the FAB I gene may be isolated by screening using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to

screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to polynucleotide assays, *inter alia*.

The polynucleotides of the invention that are oligonucleotides, including SEQ ID NOS:3 and 4, derived from the sequence of SEQ ID NO:1 may be used as PCR primers in the processes herein described to determine whether or not the *Staphylococcus aureus* genes identified herein in whole or in part are transcribed in infected tissue. The invention also provides that such sequences may also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The polynucleotides may encode a polypeptide which is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In sum, a polynucleotide of the present invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences which are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

#### **Deposited materials**

A deposit containing a *Staphylococcus aureus* WCUH 29 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (NCIMB), 23 St.



Machar Drive, Aberdeen AB2 1RY, Scotland on 11 September 1995 and assigned NCIMB Deposit No. 40771. The FAB I clone deposit is referred to herein as "the deposited clone" or as "the DNA of the deposited clone."

5 The deposited material is a strain that contains the full length FAB I DNA, referred to as "NCIMB 40771" upon deposit.

The sequence of the polynucleotides contained in the deposited material, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

10 The deposit has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposit is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

15 A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

#### **Polypeptides**

The present invention further relates to a prokaryotic FAB I polypeptide which has the deduced amino acid sequence of figure 16 [SEQ ID NO:2].

20 The invention also relates to fragments, analogs and derivatives of these polypeptides. The terms "fragment," "derivative" and "analog" when referring to the polypeptide of figure 16 [SEQ ID NO:2], means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

25 The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide. In certain preferred embodiments it is a recombinant polypeptide.

30 The fragment, derivative or analog of the polypeptide of figure 16 [SEQ ID NO:2] may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or

(iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Among the particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of FAB I set out in figure 16 [SEQ ID NO:2] variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments. Alternatively, particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of the FAB I, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of the FAB I polypeptide of figure 16 [SEQ ID NO:2] in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the FAB I. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having the amino acid sequence of figure 16 [SEQ ID NO:2] without substitutions.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as polypeptides which have at least 80% identity to the polypeptide of SEQ ID NO:2 and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of SEQ ID NO:2 and still more preferably

at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

5       Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

#### Fragments

10       Also among preferred embodiments of this aspect of the present invention are polypeptides comprising fragments of FAB I, most particularly fragments of FAB I having the amino acid set out in figure 16 [SEQ ID NO:2] and fragments of variants and derivatives of the FAB I of figure 16 [SEQ ID NO:2].

15       In this regard a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned FAB I polypeptides and variants or derivatives thereof.

Such fragments may be "free-standing," *i.e.*, not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the presently discussed  
20       fragments most preferably form a single continuous region. However, several fragments may be comprised within a single larger polypeptide. For instance, certain preferred embodiments relate to a fragment of a FAB I polypeptide of the present comprised within a precursor polypeptide designed for expression in a host and having heterologous pre and pro-polypeptide regions fused to the amino terminus of the FAB I fragment and an additional  
25       region fused to the carboxyl terminus of the fragment. Therefore, fragments in one aspect of the meaning intended herein, refers to the portion or portions of a fusion polypeptide or fusion protein derived from FAB I.

Particularly preferred fragments include those encoded by amino acids 1-20, 21-40, 41-60, 61-80, 81-100, 101-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-  
30       256 and contiguous combinations thereof.

More particularly preferred fragments include, for example, those with high homology to other sequenced structurally related elements. These include, for example, regions homologous to fragments of *E.coli fabI* including about 13 to about 25, about 32 to

about 39, about 112 to about 125, about 156 to about 196 and about 231 to about 252.

Among especially preferred fragments of the invention are truncation mutants of FAB I. Truncation mutants include FAB I polypeptides having the amino acid sequence of figure 16 [SEQ ID NO:2] or of variants or derivatives thereof, except for deletion of a continuous series of residues (that is, a continuous region, part or portion) that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or, as in double truncation mutants, deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Fragments having the size ranges set out above are preferred embodiments of truncation fragments, which are especially preferred among fragments generally. Degradation products of the polypeptides of the invention in a host cell, particularly a staphylococcus, are also preferred polypeptides.

Also preferred in this aspect of the invention are fragments characterized by structural or functional attributes of FAB I. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of FAB I.

Among the preferred fragments in this regard are those that comprise regions of FAB I that contain structural features, such as the features set out above. In this regard, the regions defined by the residues 1 to about 8, about 26 to about 34, about 39 to about 61, about 70 to about 80, about 95 to about 107, about 127 to about 139, about 162 to about 173 and about 207 to about 220 are characterized by amino acids believed to comprise alpha helix forming regions. Additionally, the regions defined by the residues about 9 to about 13, about 19 to about 25, about 62 to about 69, about 85 to about 95, about 141 to about 156, about 173 to about 188, about 220 to about 226, about 232 to about 240 are characterized by residues believed to comprise beta-sheet regions. Also, the regions defined by the residues about 80 to about 85, about 108 to about 112, about 120 to about 125, about 178 to about 183, and about 198 to about 203 are characterized by regions believed to comprise turn regions. Also, the regions defined by the residues about 80 to about 85, about 105 to about 110, about 120 to about 125, about 138 to about 143, about 145 to about 153, about 175 to about 180, about 187 to about 192, and about 240 to about 245 are characterized by amino acid compositions believed to comprise coil regions. The regions defined by the residues about 9 to about 14,

about 20 to about 36, about 86 to about 98, about 115 to about 129, about 138 to about 152, about 161 to about 179, about 185 to about 195, about 226 to about 240, about 249 to about 256 are characterized by hydrophobic amino acids. Additionally, the regions defined by the residues about 2 to about 8, about 14 to about 19, about 36 to about 86, 98 to about 115, about 130 to about 138, about 152 to about 161, about 180 to about 186, about 206 to about 226, about 239 to and about 249 are characterized by hydrophilic amino acids. Also, the regions defined by the residues about 20 to about 40, about 43 to about 62, about 72 to about 93, about 102 to about 117, about 128 to about 140, about 180 to about 220, and about 228 to about 240 are characterized by amino acid residues believed to comprise an alpha amphipathic region and the regions defined by the residues about 1 to about 10, about 27 to about 50, about 60 to about 72, about 97 to about 110, about 158 to about 191, about 208 to about 220, and about 240 to about 252 are characterized by amino acid residues believed to comprise a beta amphipathic region. Additionally, the regions defined by the residues about 38 to 58, about 77 to about 89, about 98 to about 114, about 130 to about 142, about 190 to about 204, about 208 to about 230, and about 234 to about 246 are characteristic of amino acid residues believed to comprise flexible regions. The regions defined by residues about 34 to about 62, about 98 to about 112 and about 206 to about 222 are believed to comprise surface forming regions. Additionally, the regions defined by residues about 3 to about 9, about 14 to about 20, about 25 to about 33, about 38 to about 60, about 65 to about 88, about 98 to about 114, about 120 to about 124, about 130 to about 140, about 178 to about 185, about 195 to about 228, and about 236 to about 247 are believed to comprise regions of high antigenicity. Such regions set forth above may be comprised within a larger polypeptide or may be by themselves a preferred fragment of the present invention, as discussed above. It will be appreciated that the term "about" as used in this paragraph has the meaning set out above regarding fragments in general.

Further preferred regions are those that mediate activities of FAB I. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of FAB I, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Highly preferred in this regard are fragments that contain regions that are homologs in sequence, or in position, or in both sequence and to active regions of related polypeptides, such as the related polypeptides set out in figure 16 [SEQ ID NO:2], which include E.coli Enoyl (ACP) reductase, Fab I. Among particularly preferred fragments in these

regards are truncation mutants, as discussed above. Further preferred polynucleotide fragments are those that are antigenic or immunogenic in an animal, especially in a human.

It will be appreciated that the invention also relates to, among others, polynucleotides encoding the aforementioned fragments, polynucleotides that hybridize to polynucleotides encoding the fragments, particularly those that hybridize under stringent conditions, and polynucleotides, such as PCR primers, for amplifying polynucleotides that encode the fragments. In these regards, preferred polynucleotides are those that correspondent to the preferred fragments, as discussed above.

#### Vectors, host cells, expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. For instance, polynucleotides may be introduced into host cells using well known techniques of infection, transduction, transfection, transvection and transformation. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

Thus for instance, the polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. The vector construct may be introduced into host cells by the aforementioned techniques. Generally, a plasmid vector is introduced as DNA in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. Electroporation also may be used to introduce polynucleotides into a host. If the vector is a virus, it may be packaged in vitro or introduced into a packaging cell and the packaged virus may be transduced into cells. A wide variety of techniques suitable for making polynucleotides and for introducing polynucleotides into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length in Sambrook et al. cited above, which is illustrative of the many laboratory manuals that detail these techniques. In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and

RNA into cells. The vectors, in the case of phage and viral vectors also may be and preferably are introduced into cells as packaged or encapsidated virus by well known techniques for infection and transduction. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art.

The engineered host cells can be cultured in conventional nutrient media, which may be modified as appropriate for, *inter alia*, activating promoters, selecting transformants or amplifying genes. Culture conditions, such as temperature, pH and the like, previously used with the host cell selected for expression generally will be suitable for expression of polypeptides of the present invention as will be apparent to those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present

invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques. In general, a DNA sequence for expression is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more restriction endonucleases and then joining the restriction fragments together using T4 DNA ligase. Procedures for restriction and ligation that can be used to this end are well known and routine to those of skill. Suitable procedures in this regard, and for constructing expression vectors using alternative techniques, which also are well known and routine to those skill, are set forth in great detail in Sambrook et al., (1989) *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name just a few of the well-known promoters. It will be understood that numerous promoters not mentioned are suitable for use in this aspect of the invention are well known and readily may be employed by those of skill in the manner illustrated by the discussion and the examples herein.

In general, expression constructs will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, in accordance with many commonly practiced procedures, such regions will operate by controlling transcription, such as transcription factors, repressor binding sites and termination, among others.

Vectors for propagation and expression generally will include selectable markers. Such markers also may be suitable for amplification or the vectors may contain additional markers for this purpose. In this regard, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells.



Preferred markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing *E. coli* and other prokaryotes.

The vector containing the appropriate DNA sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well known techniques suitable to expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, streptomyces and *Salmonella typhimurium* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Hosts for of a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure readily to select a host for expressing a polypeptides in accordance with this aspect of the present invention.

More particularly, the present invention also includes recombinant constructs, such as expression constructs, comprising one or more of the sequences described above. The constructs comprise a vector, such as a plasmid or viral vector, into which such a sequence of the invention has been inserted. The sequence may be inserted in a forward or reverse orientation. In certain preferred embodiments in this regard, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and there are many commercially available vectors suitable for use in the present invention.

The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("CAT") transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; *i.e.*, a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the *cat* gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available. Two such vectors are pKK232-8 and pCM7. Thus, promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene.

Among known prokaryotic promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the *E. coli* *lacI* and *lacZ* and promoters, the T3 and T7 promoters, the *gpt* promoter, the lambda PR, PL promoters and the *trp* promoter.

Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

The present invention also relates to host cells containing the above-described constructs discussed above. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell.

Introduction of the construct into the host cell can be affected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al, (1986) *BASIC METHODS IN MOLECULAR BIOLOGY*, and Sambrook et al., (1989) *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y..

Constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

5 Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., (1989) *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring  
10 Harbor, N.Y..

Generally, recombinant expression vectors will include origins of replication, a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector. Among suitable promoters are those derived from the genes that  
15 encode glycolytic enzymes such as 3-phosphoglycerate kinase ("PGK"), a-factor, acid phosphatase, and heat shock proteins, among others. Selectable markers include the ampicillin resistance gene of *E. coli* and the *trp1* gene of *S. cerevisiae*.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector.  
20 Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

25 Polynucleotides of the invention, encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located appropriately 5' to a ribosome binding site. The ribosome binding site will be 5' to the AUG that initiates translation of the  
30 polypeptide to be expressed. Generally, there will be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiating AUG. Also, generally, there will be a translation stop codon at the end of the

polypeptide and there will be a polyadenylation signal and a transcription termination signal appropriately disposed at the 3' end of the transcribed region.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals  
5 may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may  
10 be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability or to facilitate purification, among others, are  
15 familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize or purify polypeptides. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in fusion protein is thoroughly  
20 advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (see EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in assays, therapy or diagnosis, for  
25 example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, shIL-5- has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al, (1995) *Journal of Molecular Recognition*, Vol. 8 52-58 and K. Johanson et al, (1995) *The Journal of Biological Chemistry*, Vol. 270, No. 16, pp 9459-9471.

30 Suitable prokaryotic hosts for propagation, maintenance or expression of polynucleotides and polypeptides in accordance with the invention include streptococci, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhimurium*. Various species of

*Pseudomonas*, *Streptomyces*, and *Staphylococcus* are also suitable hosts in this regard. Moreover, many other hosts also known to those of skill may be employed in this regard.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning  
5 vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

10 Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, where the selected promoter is inducible it is induced by appropriate means (e.g., temperature shift or exposure to chemical inducer) and cells are cultured for an additional period.

Cells typically then are harvested by centrifugation, disrupted by physical or chemical  
15 means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can be employed for expression, as well.  
20 Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblast, described in Gluzman et al, (1981) *Cell* 23: 175. Other cell lines capable of expressing a compatible vector include for example, the C127, 3T3, CHO, HeLa, human kidney 293 and BHK cell lines.

Mammalian expression vectors may comprise an origin of replication, a suitable  
25 promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences that are necessary for expression. In certain preferred embodiments in this regard DNA sequences derived from the SV40 splice sites, and the SV40 polyadenylation sites are used for required non-transcribed genetic elements of these types.

30 FAB I polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and

lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification. this invention also provides the use of anti-FAB I antibodies for detection of FAB I during purification.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

FAB I polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of FAB I. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are illustrated further by the following discussion.

#### **Polynucleotide assays**

This invention is also related to the use of the FAB I polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of FAB I in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method that can add to, define or allow a diagnosis of a disease. Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, carrying a FAB I gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from an individual's cells and tissues, such as bone, blood, muscle, cartilage, and skin. Tissue biopsy and autopsy material is also preferred for samples from an individual to use in a diagnostic assay. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. PCR (Saiki et al., (1986) *Nature*, 324: 163-166). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding FAB I can be used to identify and analyze FAB I presence and expression. Using PCR, characterization of the strain of prokaryote present in a eukaryote, particularly a mammal, and especially a human, may be made by an analysis of the genotype of the prokaryote gene. For example, deletions and

insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridizing amplified DNA to radiolabeled FAB I RNA or alternatively, radiolabeled FAB I antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by  
5 RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a  
10 sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of  
15 alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures  
20 (see, e.g., Myers et al., (1985) *Science*, 230: 1242).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton et al., (1985) *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401).

Thus, the detection of a specific DNA sequence may be achieved by methods such as  
25 hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

30 A mutation may be ascertained for example, by a DNA sequencing assay. Samples are processed by methods known in the art to capture the RNA. First strand cDNA is synthesized from the RNA samples by adding an oligonucleotide primer consisting of sequences which hybridize to a region on the mRNA. Reverse transcriptase and

deoxynucleotides are added to allow synthesis of the first strand cDNA. Primer sequences are synthesized based on the DNA sequence of the FAB I protein of the invention. The primer sequence is generally comprised of at least 15 consecutive bases, and may contain at least 30 or even 50 consecutive bases.

5 Cells carrying mutations in the gene of the present invention may also be detected at the DNA level by a variety of techniques, to allow for serotyping, for example. Nucleic acids for diagnosis may be obtained from an individual's cells or bodily fluids, including but not limited to blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki *et al.*,  
10 (1986) *Nature*, 324:163-166) prior to analysis. RT-PCR can also be used to detect mutations. It is particularly preferred to used RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers complementary to the nucleic acid encoding FAB I can be used to identify and analyze mutations. Examples of representative primers are shown  
15 below in Table 1. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA or alternatively, radiolabeled antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

20

Table 1

## Primers used for detection of mutations in FAB I gene

<u>SEQ ID NO:</u>	<u>SEQUENCE</u>
3	5'-CGCCTCGAGATGTTAAATCTTGAAAACAAAACATATGTC-3'
4	5'-CGCGGATCCAATCAAGTCAGGTTGAAATATCCA-3'

25 The above primers may be used for amplifying FAB I DNA isolated from a sample derived from an individual. The invention also provides the primers of Table 1 with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. The primers may be used to amplify the gene isolated from the individual such that the gene may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the DNA sequence may be  
30 determined, for example, to ascertain the serotype of the organism.

Sequence differences between the reference gene and genes having mutations may be revealed by the direct DNA sequencing method. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of this method is



greatly enhanced when combined with PCR. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

- 5           Genotyping based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, *e.g.*, Myers *et al.*, (1985) *Science*, 230:1242).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, (1985) *PNAS, USA*, 85:4397-4401).

- 15           Thus, the detection of a specific DNA sequence and/or quantitation of the level of the sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (*e.g.*, Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA. The invention provides a process for diagnosing disease, particularly bacterial infections, and most particularly staphylococcal infections, comprising determining from a sample derived from an individual an increased level of expression of polynucleotide having the sequence offigure 17 [SEQ ID NO:1]. Increased expression of polynucleotide can be measured using any on of the methods well known in the art for the quantation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

#### Polypeptide assays

- 20           The present invention also relates to a diagnostic assays such as quantitative and diagnostic assays for detecting levels of FAB I protein in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting a pathological expression level of FAB I protein compared to normal control tissue samples may be used to detect the presence of bacterial

infection, for example. Assay techniques that can be used to determine levels of a protein, such as an FAB I protein of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Among these ELISAs  
5 frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to FAB I, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached a detectable reagent such as radioactive, fluorescent or enzymatic reagent, in this example horseradish peroxidase enzyme.

10 To carry out an ELISA a sample is removed from a host and incubated on a solid support, *e.g.*, a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any FAB I proteins attached to the polystyrene dish.  
15 Unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to FAB I. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to FAB I through the primary and secondary antibodies,  
20 produces a colored reaction product. The amount of color developed in a given time period indicates the amount of FAB I protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to FAB I attached to a solid support and labeled FAB I and a sample derived from form an individual are passed  
25 over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of FAB I in the sample.

#### **Antibodies**

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These  
30 antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an individual or by administering the polypeptides to an individual, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only  
5 a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma  
10 technique (Kohler, G. *et al.*, (1975) *Nature* 256: 495-497, the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, (1983) *Immunology Today* 4: 72 and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, (1985) in *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss, Inc. pp. 77-96.

Techniques described for the production of single chain antibodies (U.S. Patent No.  
15 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

Polypeptide derivatives include antigenically or immunologically equivalent  
20 derivatives which form a particular aspect of this invention.

The term "antigenically equivalent derivative" as used herein encompasses a polypeptide or its equivalent which will be specifically recognised by certain antibodies which, when raised to the protein or polypeptide according to the present invention, interfere with the immediate physical interaction between pathogen and mammalian host.

25 The term "immunologically equivalent derivative" as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the immediate physical interaction between pathogen and mammalian host.

The polypeptide, such as an antigenically or immunologically equivalent derivative  
30 or a fusion protein thereof is used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier

protein for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively a multiple antigenic peptide comprising multiple copies of the the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

5           Using the procedure of Kohler et al, (1975) *Nature* 256 , 495-497), antibody-containing cells from the immunised mammal are fused with myeloma cells to create hybridoma cells secreting monoclonal antibodies.

          The hybridomas are screened to select a cell line with high binding affinity and favorable cross reaction with other staphylococcal species using one or more of the original  
10       polypeptide and/or the fusion protein. The selected cell line is cultured to obtain the desired Mab.

          Hybridoma cell lines secreting the monoclonal antibody are another aspect of this invention.

          Alternatively phage display technology could be utilised to select antibody genes  
15       with binding activities towards the polypeptide either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-Fbp or from naive libraries (McCafferty, J. et al., (1990), *Nature* 348, 552-554; Marks, J. et al., (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. et al., (1991) *Nature* 352, 624-628).

20           The antibody should be screened again for high affinity to the polypeptide and/or fusion protein.

          The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or purify the polypeptide of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

25           The polypeptide may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused, e.g., by mechanical, traumatic, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes,  
30       such as the mouth, mammary glands, urethra or vagina.

          The polypeptides or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal

antibodies. The term antibodies also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

- 5           Antibodies generated against the polypeptides of the present invention can be obtained by direct injection of the polypeptides into an individual or by administering the polypeptides to an individual, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides.
- 10       Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

As mentioned above, a fragment of the final antibody may be prepared.

- The antibody may be either intact antibody of  $M_r$  approx 150,000 or a derivative of it, for example a Fab fragment or a Fv fragment as described in Skerra, A and Pluckthun, A (1988) *Science* 240 1038-1040. If two antigen binding domains are present each domain
- 15       may be directed against a different epitope - termed 'bispecific' antibodies.

- The antibody of the invention may be prepared by conventional means for example by established monoclonal antibody technology (Kohler, G. et al (1975), *Nature* 256, 495-497) or using recombinant means e.g. combinatorial libraries, for example as described in
- 20       Huse, W.D. et al., (1989) *Science* 246, 1275- 1281.

- Preferably the antibody is prepared by expression of a DNA polymer encoding said antibody in an appropriate expression system such as described above for the expression of polypeptides of the invention. The choice of vector for the expression system will be determined in part by the host, which may be a prokaryotic cell, such as *E. coli* (preferably
- 25       strain B) or *Streptomyces sp.* or a eukaryotic cell, such as a mouse C127, mouse myeloma, human HeLa, Chinese hamster ovary, filamentous or unicellular fungi or insect cell. The host may also be a transgenic animal or a transgenic plant, for example as described in Hiatt, A et al, (1989) *Nature* 34, 76-78. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses, derived from, for example, baculoviruses and vaccinia.

- 30       The Fab fragment may also be prepared from its parent monoclonal antibody by enzyme treatment, for example using papain to cleave the Fab portion from the Fc portion.

Preferably the antibody or derivative thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be 'humanised'; where the complementarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones, P. et al. (1986), *Nature* 321, 522-525 or Tempest et al., (1991) *Biotechnology* 9, 266-273.

The modification need not be restricted to one of 'humanisation': other primate sequences (for example Newman, R. et al., 1992, *Biotechnology* 10, 1455-1460) may also be used.

10 The humanised monoclonal antibody, or its fragment having binding activity, form a particular aspect of this invention.

This invention provides a method of screening drugs to identify those which interfere with the interaction of the FAB I protein or active fragment to mammalian cells, the method comprising incubating a mammalian cell or membrane preparation with labeled polypeptide in the presence of the drug and measuring the ability of the drug to block this interaction.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., (1992) *Hum Mol Genet*, 1:363, Manthorpe et al., (1963) *Hum. Gene Ther.* 4, 419), delivery of DNA complexed with specific protein carriers (Wu, et al, (1989) *J Biol Chem* 264, 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, PNAS.1986:83,9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., (1989) *Science* 243, 375), particle bombardment (Tang et al., (1992) *Nature* 356:152, Eisenbraun et al., (1993) *DNA Cell Biol* 12:791) and *in vivo* infection using cloned retroviral vectors (Seeger et al., (1984) *PNAS* 81, 5849). Suitable promoters for muscle transfection include CMV, RSV, SRa, actin, MCK, alpha globin, adenovirus and dihydrofolate reductase.

Thus, among others, antibodies against FAB I may be employed to inhibit FAS or FAB I enzymatic activity or FAB I expression.

30 FAB I may also be employed to inhibit infections including, but not limited to infections of upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g. empyema, lung abscess), cardiac (e.g. infective

endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (e.g. epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis), and bone and joint (e.g. septic arthritis, osteomyelitis).

FAB I may also be employed to treat bacterial infection, such as, for example, infections of upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g. empyema, lung abscess), cardiac (e.g. infective endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (e.g. epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis), and bone and joint (e.g. septic arthritis, osteomyelitis).

#### FAB I binding molecules and assays

This invention also provides a method for identification of molecules, such as binding molecules, that bind FAB I. Genes encoding proteins that bind FAB I, such as binding proteins, can be identified by numerous methods known to those of skill in the art. Examples of such methods are described in many laboratory manuals such as, for instance, Coligan *et al.*, (1991) *Current Protocols in Immunology* 1(2): Chapter 5.

For instance, expression cloning may be employed for this purpose. To this end, to isolate genes in an individual which are responsive to FAB I, polyadenylated RNA is prepared from a cell responsive to FAB I, a cDNA library is created from this RNA, the library is divided into pools and the pools are transfected individually into cells that are not responsive to FAB I. The transfected cells then are exposed to labeled FAB I. (FAB I can be labeled by a variety of well-known techniques including standard methods of radio-iodination or inclusion of a recognition site for a site-specific protein kinase.) Following exposure, the cells are fixed and binding of FAB I is determined. These procedures conveniently are carried out on glass slides.

Alternatively a labeled ligand can be photoaffinity linked to a cell extract, such as a membrane or a membrane extract, prepared from cells that express a molecule that it binds, such as a binding molecule. Cross-linked material is resolved by polyacrylamide gel

electrophoresis ("PAGE") and exposed to X-ray film. The labeled complex containing the ligand-binding can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing can be used to design unique or degenerate oligonucleotide probes to screen cDNA libraries to identify genes  
5 encoding the putative binding molecule.

Polypeptides of the invention also can be used to assess FAB I binding capacity of FAB I binding molecules, such as binding molecules, in cells or in cell-free preparations.

#### Antagonists and Agonists - Assays and Molecules

Polypeptides and polynucleotides of the invention may also be used to assess the  
10 binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, *e.g.*, Coligan *et al.*, *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

Polypeptides and polynucleotides of the present invention are responsible for many  
15 biological functions, including many disease states, in particular the Diseases hereinbefore mentioned. It is therefore desirable to devise screening methods to identify compounds which stimulate or which inhibit the function of the polypeptide or polynucleotide. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those which stimulate or which inhibit the function of a polypeptide or polynucleotide of the  
20 invention, as well as related polypeptides and polynucleotides. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes for such Diseases as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists, antagonists or inhibitors so-identified may be natural or modified substrates, ligands, receptors,  
25 enzymes, etc., as the case may be, of FAB I polypeptides and polynucleotides; or may be structural or functional mimetics thereof (see Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991)).

The screening methods may simply measure the binding of a candidate compound to the polypeptide or polynucleotide, or to cells or membranes bearing the polypeptide or  
30 polynucleotide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test



whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring FAB I polypeptide and/or polynucleotide activity in the mixture, and comparing the FAB I polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and FAB I polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of FAB I polypeptides or polynucleotides, particularly those compounds that are bacteristatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising FAB I polypeptide and a labeled substrate or ligand

of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a FAB I agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the FAB I polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of FAB I polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in FAB I polynucleotide or polypeptide activity, and binding assays known in the art.

Polypeptides of the invention may be used to identify membrane bound or soluble receptors, if any, for such polypeptide, through standard receptor binding techniques known in the art. These techniques include, but are not limited to, ligand binding and crosslinking assays in which the polypeptide is labeled with a radioactive isotope (for instance,  $^{125}\text{I}$ ), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (*e.g.*, cells, cell membranes, cell supernatants, tissue extracts, bodily materials). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide which compete with the binding of the polypeptide to its receptor(s), if any. Standard methods for conducting such assays are well understood in the art.

The fluorescence polarization value for a fluorescently-tagged molecule depends on the rotational correlation time or tumbling rate. Protein complexes, such as formed by FAB I polypeptide associating with another FAB I polypeptide or other polypeptide, labeled to comprise a fluorescently-labeled molecule will have higher polarization values than a fluorescently labeled monomeric protein. It is preferred that this method be used to characterize small molecules that disrupt polypeptide complexes.

Fluorescence energy transfer may also be used characterize small molecules that interfere with the formation of FAB I polypeptide dimers, trimers, tetramers or higher order structures, or structures formed by FAB I polypeptide bound to another polypeptide. FAB

I polypeptide can be labeled with both a donor and acceptor fluorophore. Upon mixing of the two labeled species and excitation of the donor fluorophore, fluorescence energy transfer can be detected by observing fluorescence of the acceptor. Compounds that block dimerization will inhibit fluorescence energy transfer.

5           Surface plasmon resonance can be used to monitor the effect of small molecules on FAB I polypeptide self-association as well as an association of FAB I polypeptide and another polypeptide or small molecule. FAB I polypeptide can be coupled to a sensor chip at low site density such that covalently bound molecules will be monomeric. Solution protein can then be passed over the FAB I polypeptide-coated surface and specific binding  
10 can be detected in real-time by monitoring the change in resonance angle caused by a change in local refractive index. This technique can be used to characterize the effect of small molecules on kinetic rates and equilibrium binding constants for FAB I polypeptide self-association as well as an association of FAB I polypeptide and another polypeptide or small molecule.

15           A scintillation proximity assay may be used to characterize the interaction between an association of FAB I polypeptide with another FAB I polypeptide or a different polypeptide. FAB I polypeptide can be coupled to a scintillation-filled bead. Addition of radio-labeled FAB I polypeptide results in binding where the radioactive source molecule is in close proximity to the scintillation fluid. Thus, signal is emitted upon FAB I polypeptide  
20 binding and compounds that prevent FAB I polypeptide self-association or an association of FAB I polypeptide and another polypeptide or small molecule will diminish signal.

ICS biosensors have been described by AMBRI (Australian Membrane Biotechnology Research Institute). They couple the self-association of macromolecules to the closing of gramicidin-facilitated ion channels in suspended membrane bilayers and  
25 hence to a measurable change in the admittance (similar to impedance) of the biosensor. This approach is linear over six decades of admittance change and is ideally suited for large scale, high through-put screening of small molecule combinatorial libraries.

In other embodiments of the invention there are provided methods for identifying compounds which bind to or otherwise interact with and inhibit or activate an activity or  
30 expression of a polypeptide and/or polynucleotide of the invention comprising: contacting a polypeptide and/or polynucleotide of the invention with a compound to be screened under conditions to permit binding to or other interaction between the compound and the polypeptide

and/or polynucleotide to assess the binding to or other interaction with the compound, such binding or interaction preferably being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the polypeptide and/or polynucleotide with the compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an activity or expression of the polypeptide and/or polynucleotide by detecting the presence or absence of a signal generated from the binding or interaction of the compound with the polypeptide and/or polynucleotide.

Another example of an assay for FAB I agonists is a competitive assay that combines FAB I and a potential agonist with FAB I-binding molecules, recombinant FAB I binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. FAB I can be labeled, such as by radioactivity or a colorimetric compound, such that the number of FAB I molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

A further example of a screen is to detect the reduction of crotonyl-CoA by measuring the consumption of NADH. Crotonyl-ACP may also be used in place of crotonyl-CoA in such a screen. Test compounds are added to the reaction mix to determine the effect on the reduction of crotonyl-CoA or crotonyl-ACP. Agonists can be identified if the level of reduction is increased and antagonists can be identified if the level of reduction is decreased. Diazaborine or palmitoyl CoA may be used as a positive control for antagonism. It is preferred that these screens be used in a highthroughput assay using a microtitre plate format and a plate reader.

The following observations relating to Fab I enzyme kinetics are useful in the methods of the inventions and in other aspects of the invention:

1. Apo-ACP is uncompetitive versus NADH ( $K_i(\text{app})$  and is proportional to the square of [CCA]).
2. Apo-ACP is competitive versus crotonoyl CoA and induces negative cooperativity with respect to CCA binding.
3. Stereochemical and steady-state kinetic studies have been carried out on purified *S. aureus* Fab I.
4. The stereochemical course for the *S. aureus* Fab I is identical to that reported for the *E. coli* enzyme.

5. In contrast to the *E. coli* enzyme which uses only NADH, *S. aureus* Fab I uses both NADH and NADPH as substrates. However, the  $K_{m_{app}}$  for NADH is approximately 30-fold higher than for NADPH.
  6. With NADH and crotonoyl CoA as substrates, Fab I displays a sequential kinetic mechanism.
  7. Kinetic parameters vary little over the pH range of 5.0-7.0.
  8. Increasing inhibition is observed with saturated fatty acyl CoA's of increasing chain length suggestive of a possible feedback regulatory mechanism *in vivo*.
  9. Palmitoyl CoA appears to be a competitive inhibitor with respect to crotonoyl CoA.
  10. Slope replots are proportional to  $[I]^3$ , suggesting that inhibition is effected through the binding of multiple palmitoyl CoA molecules to Fab I.
  10. Apo-ACP is uncompetitive versus NADH and competitive versus crotonoyl CoA while inducing negative cooperativity in the binding of CCA. This indicates an oligomeric quaternary structure, particularly, but not limited to, a dimer or tetramer.
  11. Studies using apo-ACP and the pseudo-product inhibitors beta-NADP<sup>+</sup> and palmitoyl CoA indicate an ordered addition of substrates (NADH binding first).
  12. The uncompetitive inhibition of apo-ACP versus NADH suggests that prior binding of the pyridine nucleotide is *required* for ACP binding.
  13. The *S. aureus* enzyme had similar affinity to the *E. coli* enzyme for the crotonoyl CoA substrate, yet the *E. coli* enzyme possessed up to 70 fold greater affinity for NADH, which was suprising.
  14. His<sub>10</sub> tagged *S. aureus* Fab I displays distinct kinetic characteristics as compared to native Fab I enzyme.
  15. *S. aureus* Fab I is inhibited by fatty acyl CoAs indicating that this enzyme is subject to feed back inhibition by long chain acyl-acyl carrier proteins *in vivo*.
- Potential antagonists of the invention include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing FAB I-induced activities, thereby preventing the action or expression of FAB I polypeptides and/or polynucleotides by excluding FAB I polypeptides and/or polynucleotides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of FAB I.

Small organic molecules of the invention preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. An embodiment provides compounds that were not published prior to the filing date of this application. Other examples of potential polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Certain of the polypeptides of the invention are biomimetics, functional mimetics of the natural FAB I polypeptide. These functional mimetics may be used for, among other things, antagonizing the activity of FAB I polypeptide or as an antigen or immunogen in a manner described elsewhere herein. Functional mimetics of the polypeptides of the invention include but are not limited to truncated polypeptides. For example, preferred functional mimetics include, a polypeptide comprising the polypeptide sequence set forth in SEQ ID NO:2 lacking 20, 30, 40, 50, 60, 70 or 80 amino- or carboxy-terminal amino acid residues, including fusion proteins comprising one or more of these truncated sequences. Polynucleotides encoding each of these functional mimetics may be used as expression cassettes to express each mimetic polypeptide. It is preferred that these cassettes comprise 5' and 3' restriction sites to allow for a convenient means to ligate the cassettes together when desired. It is further preferred that these cassettes comprise gene expression signals known in the art or described elsewhere herein.

Thus, in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for a polypeptide and/or

polynucleotide of the present invention; or compounds which decrease or enhance the production of such polypeptides and/or polynucleotides, which comprises: (a) a polypeptide and/or a polynucleotide of the present invention; (b) a recombinant cell expressing a polypeptide and/or polynucleotide of the present invention; (c) a cell membrane expressing a polypeptide and/or polynucleotide of the present invention; or (d) antibody to a polypeptide and/or polynucleotide of the present invention; which polypeptide is preferably that of SEQ ID NO:2, and which polynucleotide is preferably that of SEQ ID NO:1.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

It will be readily appreciated by the skilled artisan that a polypeptide and/or polynucleotide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of the polypeptide and/or polynucleotide, by: (a) determining in the first instance the three-dimensional structure of the polypeptide and/or polynucleotide, or complexes thereof; (b) deducing the three-dimensional structure for the likely reactive site(s), binding site(s) or motif(s) of an agonist, antagonist or inhibitor; (c) synthesizing candidate compounds that are predicted to bind to or react with the deduced binding site(s), reactive site(s), and/or motif(s); and (d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors. It will be further appreciated that this will normally be an iterative process, and this iterative process may be performed using automated and computer-controlled steps.

In a further aspect, the present invention provides methods of treating abnormal conditions such as, for instance, a Disease, related to either an excess of, an under-expression of, an elevated activity of, or a decreased activity of FAB I polypeptide and/or polynucleotide.

If the expression and/or activity of the polypeptide and/or polynucleotide is in excess, several approaches are available. One approach comprises administering to an individual in need thereof an inhibitor compound (antagonist) as herein described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function and/or expression of the polypeptide and/or polynucleotide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of the polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous polypeptide and/or polynucleotide may be administered.

Typical examples of such competitors include fragments of the FAB I polypeptide and/or polypeptide.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and  
5 various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this  
10 invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

15 In still another approach, expression of the gene encoding endogenous FAB I polypeptide can be inhibited using expression blocking techniques. This blocking may be targeted against any step in gene expression, but is preferably targeted against transcription and/or translation. An examples of a known technique of this sort involve the use of antisense sequences, either internally generated or separately administered (see, for example,  
20 O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Alternatively, oligonucleotides which form triple helices with the gene can be supplied (see, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360). These oligomers can be administered *per se* or the relevant oligomers can be  
25 expressed *in vivo*.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or  
30 other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.



The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of  
5 adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial FAB I proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections  
10 initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided FAB I agonists and antagonists, preferably bacteristatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

15 The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The antagonists may be employed for instance to inhibit diseases including, but not limited to infections of upper respiratory tract (e.g. otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g. empyema, lung abscess), cardiac (e.g. infective  
20 endocarditis), gastrointestinal (e.g. secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g. cerebral abscess), eye (e.g. blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (e.g. epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g. impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis), and bone and  
25 joint (e.g. septic arthritis, osteomyelitis).

The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

Each of the DNA sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein upon expression can be  
30 used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

In view of the conservation in the amino acid sequence of FAB I with known enzymes in *H.influenzae*, *E.coli* and *S. typhimurium*, an antibacterial targeted at FAB I agonist and antagonist compounds provided by the invention are believed to be active against a wide variety of Gram negative and positive organisms. In addition, a Fab I homolog (InhA) has been identified in *Mycobacterium tuberculosis*. Therefore, an antibacterial provided by the invention, targeted at FAB I, may also have anti -mycobacterial activity. Further, since Diazaborine derivatives inhibit LPS biosynthesis (Lam *et al* (1987) *J. Antimicrob. Chemother.* 20 37-45) and LPS is a known virulence factor, the invention provides antibacterial compounds targeted at FAB I that have enhanced activity *in vivo*.

*Helicobacter pylori* (herein "*H. pylori*") bacteria infect the stomachs of over one-third of the world's population causing stomach cancer, ulcers, and gastritis (International Agency for Research on Cancer (1994) *Schistosomes, Liver Flukes and Helicobacter Pylori* (International Agency for Research on Cancer, Lyon, France, <http://www.uicc.ch/ecp/ecp2904.htm>). Moreover, the International Agency for Research on Cancer recently recognized a cause-and-effect relationship between *H. pylori* and gastric adenocarcinoma, classifying the bacterium as a Group I (definite) carcinogen. Preferred antimicrobial compounds of the invention (agonists and antagonists of FAB I polypeptides and/or polynucleotides) found using screens provided by the invention, or known in the art, particularly narrow-spectrum antibiotics, should be useful in the treatment of *H. pylori* infection. Such treatment should decrease the advent of *H. pylori*-induced cancers, such as gastrointestinal carcinoma. Such treatment should also prevent, inhibit and/or cure gastric ulcers and gastritis.

#### Compositions

The invention also relates to compositions comprising the polynucleotide or the polypeptides discussed above or the agonists or antagonists. Thus, the polypeptides of the present invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

### Kits

The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the product for human administration.

Also provided by the invention are diagnostic kits for the FAB I gene and homologs will enable the directed therapy of a FAB I directed antibacterial. DNA hybridisation or protein (monoclonal antibody) based kits for the identification of Fab I and homologs of FAB I from different species. Such a kit could also detect mutations in the FAB I gene /protein and its homologs.

### Administration

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

The pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific indication or indications. In general, the compositions are administered in an amount of at least about 10 µg/kg body weight. In most cases they will be administered in an amount not in excess of about 8 mg/kg body weight per day. Preferably, in most cases, dose is from about 10 µg/kg to about 1 mg/kg body weight, daily. It will be appreciated that optimum dosage will be determined by standard methods for each treatment modality and indication, taking into account the indication, its severity, route of administration, complicating conditions and the like.

In therapy or as a prophylactic, the active agent may be administered to a individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug

penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

For administration to human individuals, it is expected that the daily dosage level of the active agent will be from 0.01 to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters, etc.

The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent staphylococcal wound infections.

Many orthopaedic surgeons consider that individuals with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteraemia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis. Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before

insertion. The active agent will preferably be present at a concentration of 1µg/ml to 10mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response.

- 5           A suitable unit dose for vaccination is 0.5-5ug/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks.

With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

- 10           The antibodies described above may also be used as diagnostic reagents to detect the presence of bacteria containing the FAB I protein.

In order to facilitate understanding of the following example certain frequently occurring methods and/or terms will be described.

#### EXAMPLES

- 15           The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplification's, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Certain terms used herein are explained in the foregoing glossary.

- 20           All examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., (1989) *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y..

25           All parts or amounts set out in the following examples are by weight, unless otherwise specified.

- 30           Unless otherwise stated size separation of fragments in the examples below was carried out using standard techniques of agarose and polyacrylamide gel electrophoresis ("PAGE") in Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and numerous other references such as, for instance, by Goeddel et al., (1980) *Nucleic Acids Res.* 8: 4057.

Unless described otherwise, ligations were accomplished using standard buffers, incubation temperatures and times, approximately equimolar amounts of the DNA fragments to be ligated and approximately 10 units of T4 DNA ligase ("ligase") per 0.5 µg of DNA.

**Example 1 Isolation and sequencing of *S. aureus* FAB I gene**

5 The polynucleotide having the DNA sequence given in SEQ ID NO:1 was obtained from the sequencing of a library of clones of chromosomal DNA of *S.aureus* WCUH 29in *E.coli*. It has been demonstrated by the process herein described that it is transcribed *in vivo* in an established infection of *S.aureus* WCUH 29in a mouse model of infection.

To obtain the polynucleotide encoding FAB I protein using the DNA sequence  
10 given in SEQ ID NO:1 typically a library of clones of chromosomal DNA of *S.aureus* WCUH 29in *E.coli* or some other suitable host is probed with a radiolabelled oligonucleotide, preferably a 17mer or longer, derived from the partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using high stringency washes. By sequencing the individual clones thus identified with sequencing primers  
15 designed from the original sequence it is then possible to extend the sequence in both directions to determine the full gene sequence. Conveniently such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., (1989) *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold  
20 Spring Harbor, New York. (see Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70).

In some cases the sequencing data from two or more clones containing overlapping *S.aureus*WCUH 29 DNA was used to construct the contiguous DNA sequence in SEQ ID NO:1. Libraries may be prepared Libraries may be prepared by routine methods, for  
25 example: *Methods 1 and 2*

Total cellular DNA is isolated from *Staphylococcus aureus* strain WCUH 29(NCIMB 40771) according to standard procedures and size-fractionated by either of two methods.

**Method 1**

30 Total cellular DNA is mechanically sheared by passage through a needle in order to size-fractionate according to standard procedures. DNA fragments of up to 11kbp in size are rendered blunt by treatment with exonuclease and DNA polymerase, and EcoRI linkers added. Fragments are ligated into the vector Lambda ZapII that has been cut with EcoRI,

the library packaged by standard procedures and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

#### **Method 2**

5 Total cellular DNA is partially hydrolysed with a combination of four restriction enzymes (RsaI, PstI, AluI and Bsh1235I) and size-fractionated according to standard procedures. EcoRI linkers are ligated to the DNA and the fragments then ligated into the vector Lambda ZapII that have been cut with EcoRI, the library packaged by standard procedures, and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

#### **10 Example 2 FAB I enzyme activity analysis**

The activity of FAB I protein may be measured using either crotonoyl-CoA or crotonoyl -ACP as a substrate (Bergler, et al, (1994), *J.Biol.Chem.* 269, 5493-5496) and monitoring the decrease in absorbance at 340nm due to the consumption of NADH. Crotonoyl-ACP (Km 22uM) is a better substrate than crotonoyl-CoA (Km 2.7mM),  
15 crotonoyl-CoA is available from Sigma (C6146). A diazaborine derivative may be used as a positive control, this should be readily available via a 2 step synthesis with publicly available starting materials using methods known in the art. Test compounds may be added to this assay to determine whether they agonize or antagonize enzymatic activity.

#### **Example 3 Kinetic Characterization of Fab I**

20 Crotonoyl CoA was used as the substrate for Fab I and all activity measurements were based on a decrease in absorbance at 340 nm due to the utilization of NADH (Figure 1). All assays were performed with 100 mM NaADA, pH 6.5 buffer. Acyl-CoA inhibition data was performed in the presence of 1mM crotonoyl CoA and 1mM NADH.

#### **Example 4 Kinetic characterization of Fab I from *S.aureus***

25 Detailed kinetic analysis of the His<sub>10</sub> tagged enzyme demonstrated significant evidence for substrate inhibition (by both substrates) and a drastic change from a sequential to and apparent ping-pong mechanism between pH 6.5 and 6.25. However, studies performed at pH 6.0-7.0 with the native enzyme illustrated a sequential mechanistic pattern and kinetic parameters resulting from these studies indicated no overt change in either  
30 magnitude or kinetic mechanism. Thus the His<sub>10</sub> tagged Fab I behaved distinctly from native Fab I enzyme.

The kinetic parameters for native *S.aureus* Fab I are shown in Table 2.

TABLE 2. Kinetic constants for *S.aureus* Fab I

Fab I	K <sub>NADH</sub>	K <sub>CCA</sub>	K <sub>iNADH</sub>
<i>S.aureus</i>	0.7 mM	2.5 mM	0.2 mM.
<i>E.coli</i>	0.01mM	2.7mM*	

\* Bergler, H., *et al.* (1994) J. Biol. Chem. 269, 5493-6.

- 5 Native *S. aureus* Fab I exhibited a flat pH optimum between 5 and 7 over which the V<sub>m</sub> and the V<sub>m</sub>/K<sub>m</sub>'s for the substrates crotonoyl CoA (CCA) and NADH were relatively constant.

Native *S. aureus* Fab I was susceptible to inhibition by fatty acyl CoAs. The potency of inhibition increased with chain length (Table 3).

Fatty Acid	Current Results Mean IC <sub>50</sub> (uM) (n=2)
Acetyl	>>1000
n-Butyryl	>>1000
n-Hexanoyl	576
n-Octanoyl	248
n-Decanoyl	226
Lauroyl	48.4
Myristoyl	23.1
Palmitoyl	10.7

10

TABLE 3. Inhibition of non his tagged Fab I by saturated fatty acyl CoA's

- 15 It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

- Each reference cited herein is hereby incorporated by reference in its entirety. Moreover, each patent application to which this application claims priority is hereby  
20 incorporated by reference in its entirety.



**What is claimed is:**

1. An antagonist that inhibits or an agonist that activates an activity a polypeptide selected from the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4,  
5 and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, wherein said activity is selected from the group consisting of:
  - uncompetitive inhibition by Apo-ACP versus NADH (K<sub>i</sub>(app);
  - competitive inhibition by Apo-ACP versus crotonoyl CoA;
  - induction of negative cooperativity with respect to CCA binding;
  - 10 use of NADH and NADPH as substrates by Fab I;
  - binding of NADH and NADPH by FabI;
  - oxidation of NADH and NADPH by FabI;
  - ratio of K<sub>m</sub>app for NADH as compared to NADPH;
  - use of NADH and crotonoyl CoA as substrates by Fab I in a sequential
  - 15 kinetic mechanism;
  - sequential binding of NADH and crotonoyl CoA by Fab I;
  - increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length;
  - feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
  - 20 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
  - competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
  - binding of multiple palmitoyl CoA molecules to Fab I;
  - negative cooperativity in the binding of CCA;
  - 25 formation of an dimeric quaternary structure;
  - formation of an tetrameric quaternary structure;
  - formation of an oligomeric quaternary structure;
  - binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl coA; and
  - 30 NADH binding to Fab I prior to or simultaneous with ACP binding.
2. A method for the treatment of an individual having need to inhibit or activate Fab I polypeptide comprising the steps of: administering to the individual a antibacterially

- effective amount of an antagonist that inhibits or an agonist that activates an activity of a polypeptide selected from the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4,
- 5 wherein said activity is selected from the group consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );
  - competitive inhibition by Apo-ACP versus crotonoyl CoA;
  - induction of negative cooperativity with respect to CCA binding;
  - use of NADH and NADPH as substrates by Fab I;
  - 10 binding of NADH and NADPH by FabI;
  - oxidation of NADH and NADPH by FabI;
  - ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;
  - use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
  - 15 sequential binding of NADH and crotonoyl CoA by Fab I;
  - increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length;
  - feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
  - competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
  - 20 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
  - binding of multiple palmitoyl CoA molecules to Fab I;
  - negative cooperativity in the binding of CCA;
  - formation of a dimeric quaternary structure;
  - 25 formation of a tetrameric quaternary structure;
  - formation of an oligomeric quaternary structure;
  - binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl coA; and
  - NADH binding to Fab I prior to or simultaneous with ACP binding.
- 30 3. A method for the treatment of an individual infected with a bacteria comprising the steps of administering to the individual an antibacterially effective amount of an antagonist that inhibits or an agonist that activates an activity of a polypeptide selected from

the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90% identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, wherein said activity is selected from the group consisting of:

- 5           uncompetitive inhibition by Apo-ACP versus NADH (K<sub>i</sub>(app);  
competitive inhibition by Apo-ACP versus crotonoyl CoA;  
induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
binding of NADH and NADPH by FabI;
- 10          oxidation of NADH and NADPH by FabI;  
ratio of K<sub>m</sub>app for NADH as compared to NADPH;  
use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
kinetic mechanism;  
sequential binding of NADH and crotonoyl CoA by Fab I;
- 15          increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
chain length; feedback regulatory mechanism of Fab I by saturated fatty  
acyl CoA's;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation
- 20          through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
negative cooperativity in the binding of CCA;  
formation of an dimeric quaternary structure;  
formation of an tetrameric quaternary structure;
- 25          formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl  
coA; and  
NADH binding to Fab I prior to or simultaneous with ACP binding.
4.          The method of claim 3 wherein said bacteria is selected from the group
- 30          consisting of a member of the genus *Staphylococcus*, *Staphylococcus aureus*, a member of the  
genus *Streptococcus*, and *Streptococcus pneumoniae*.

5. A method for the treatment of an individual having need to inhibit or activate Fab I polypeptide comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits or an agonist that activates an activity of Fab I selected from the group consisting of:

- 5 uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );
- competitive inhibition by Apo-ACP versus crotonoyl CoA;
- induction of negative cooperativity with respect to CCA binding;
- use of NADH and NADPH as substrates by Fab I;
- binding of NADH and NADPH by FabI;
- 10 oxidation of NADH and NADPH by FabI;
- ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;
- use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
- sequential binding of NADH and crotonoyl CoA by Fab I;
- 15 increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation
- 20 through binding of multiple palmitoyl CoA molecules to Fab I;
- binding of multiple palmitoyl CoA molecules to Fab I;
- negative cooperativity in the binding of CCA;
- formation of a dimeric quaternary structure;
- formation of a tetrameric quaternary structure;
- 25 formation of an oligomeric quaternary structure;
- binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl coA; and
- NADH binding to Fab I prior to or simultaneous with ACP binding.

6. A method for the treatment of an individual infected with a bacteria
- 30 comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits or an agonist that activates that activates an activity of Fab I selected from the group consisting of:

- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );  
 competitive inhibition by Apo-ACP versus crotonoyl CoA;  
 induction of negative cooperativity with respect to CCA binding;  
 use of NADH and NADPH as substrates by Fab I;  
 5 binding of NADH and NADPH by FabI;  
 oxidation of NADH and NADPH by FabI;  
 ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;  
 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
 kinetic mechanism;  
 10 sequential binding of NADH and crotonoyl CoA by Fab I;  
 increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
 chain length; feedback regulatory mechanism of Fab I by saturated fatty  
 acyl CoA's;  
 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
 15 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
 through binding of multiple palmitoyl CoA molecules to Fab I;  
 binding of multiple palmitoyl CoA molecules to Fab I;  
 negative cooperativity in the binding of CCA;  
 formation of an dimeric quaternary structure;  
 20 formation of an tetrameric quaternary structure;  
 formation of an oligomeric quaternary structure;  
 binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl  
 coA; and  
 NADH binding to Fab I prior to or simultaneous with ACP binding.
- 25 7. The method of claim 6 wherein said bacteria is selected from the group  
 consisting of: a member of the genus *Staphylococcus*, *Staphylococcus aureus*, a member of  
 the genus *Streptococcus*, and *Streptococcus pneumoniae*.
8. A method for the treatment of an individual infected by *Streptococcus*  
*pneumoniae* comprising the steps of administering to the individual a antibacterially effective  
 30 amount of an antagonist that inhibits or an agonist that activates an activity of *Streptococcus*  
*pneumoniae* Fab I selected from the group consisting of:  
 uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );

- competitive inhibition by Apo-ACP versus crotonoyl CoA;  
 induction of negative cooperativity with respect to CCA binding;  
 use of NADH and NADPH as substrates by Fab I;  
 binding of NADH and NADPH by FabI;  
 5 oxidation of NADH and NADPH by FabI;  
 ratio of  $K_{mapp}$  for NADH as compared to NADPH;  
 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
 kinetic mechanism;  
 sequential binding of NADH and crotonoyl CoA by Fab I;  
 10 increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
 chain length; feedback regulatory mechanism of Fab I by saturated fatty  
 acyl CoA's;  
 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
 competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
 15 through binding of multiple palmitoyl CoA molecules to Fab I;  
 binding of multiple palmitoyl CoA molecules to Fab I;  
 negative cooperativity in the binding of CCA;  
 formation of an dimeric quaternary structure;  
 formation of an tetrameric quaternary structure;  
 20 formation of an oligomeric quaternary structure;  
 binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
 coA; and  
 NADH binding to Fab I prior to or simultaneous with ACP binding.
9. An antagonist that inhibits an activity of a polypeptide selected from the  
 25 group consisting of: a polypeptide comprising an amino acid sequence which is at least 90%  
 identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an  
 amino acid sequence as set forth in SEQ ID NO:2 or 4, wherein said activity is selected from  
 the group consisting of:  
 uncompetitive inhibition by Apo-ACP versus NADH ( $K_{i(app)}$ );  
 30 competitive inhibition by Apo-ACP versus crotonoyl CoA;  
 induction of negative cooperativity with respect to CCA binding;  
 use of NADH and NADPH as substrates by Fab I;

- binding of NADH and NADPH by FabI;
  - oxidation of NADH and NADPH by FabI;
  - ratio of  $K_{app}$  for NADH as compared to NADPH;
  - use of NADH and crotonoyl CoA as substrates by Fab I in a sequential
  - 5 kinetic mechanism;
  - sequential binding of NADH and crotonoyl CoA by Fab I;
  - increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
  - 10 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;
  - competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;
  - binding of multiple palmitoyl CoA molecules to Fab I;
  - negative cooperativity in the binding of CCA;
  - 15 formation of an dimeric quaternary structure;
  - formation of an tetrameric quaternary structure;
  - formation of an oligomeric quaternary structure;
  - binding of Fab I by pseudo-product inhibitors beta-NADP<sup>+</sup> or palmitoyl coA; and
  - 20 NADH binding to Fab I prior to or simultaneous with ACP binding.
10. A method for the treatment of an individual having need to inhibit Fab I polypeptide comprising the steps of administering to the individual a antibacterially effective amount of an antagonist that inhibits an activity of a polypeptide selected from the group consisting of: a polypeptide comprising an amino acid sequence which is at least 90%
- 25 identical to the amino acid sequence of SEQ ID NO:2 or 4, and a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2 or 4, , wherein said activity is selected from the group consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH ( $K_{i(app)}$ );
  - competitive inhibition by Apo-ACP versus crotonoyl CoA;
  - 30 induction of negative cooperativity with respect to CCA binding;
  - use of NADH and NADPH as substrates by Fab I;
  - binding of NADH and NADPH by FabI;

- oxidation of NADH and NADPH by FabI;  
ratio of  $K_{mapp}$  for NADH as compared to NADPH;  
use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;
- 5 sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing chain length; feedback regulatory mechanism of Fab I by saturated fatty acyl CoA's;
- 10 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
negative cooperativity in the binding of CCA;  
formation of an dimeric quaternary structure;
- 15 formation of an tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl coA; and  
NADH binding to Fab I prior to or simultaneous with ACP binding.
- 20 11. A method for inhibiting an activity of Fab I polypeptide comprising the steps of contacting a composition comprising said polypeptide with an effective amount of an antagonist that inhibits an activity of Fab I, wherein said activity is selected from the group consisting of:
- 25 uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(app)$ );  
competitive inhibition by Apo-ACP versus crotonoyl CoA;  
induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
binding of NADH and NADPH by FabI;  
oxidation of NADH and NADPH by FabI;
- 30 ratio of  $K_{mapp}$  for NADH as compared to NADPH;  
use of NADH and crotonoyl CoA as substrates by Fab I in a sequential kinetic mechanism;



- sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
chain length; feedback regulatory mechanism of Fab I by saturated fatty  
acyl CoA's;
- 5 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
negative cooperativity in the binding of CCA;
- 10 formation of an dimeric quaternary structure;  
formation of a tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
coA; and
- 15 NADH binding to Fab I prior to or simultaneous with ACP binding.
12. A method for inhibiting an activity of Fab I, wherein said activity is selected  
from the group consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH ( $K_i(\text{app})$ );  
competitive inhibition by Apo-ACP versus crotonoyl CoA;
- 20 induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
binding of NADH and NADPH by FabI;  
oxidation of NADH and NADPH by FabI;  
ratio of  $K_{\text{mapp}}$  for NADH as compared to NADPH;
- 25 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
kinetic mechanism;  
sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
chain length; feedback regulatory mechanism of Fab I by saturated fatty  
acyl CoA's;
- 30 competitive inhibition by palmitoyl CoA versus crotonoyl CoA;

- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
negative cooperativity in the binding of CCA;  
5 formation of an dimeric quaternary structure;  
formation of an tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
coA; and  
10 NADH binding to Fab I prior to or simultaneous with ACP binding.
13. The method of claim 12 wherein said bacteria is selected from the group  
consisting of: a member of the genus *Staphylococcus*, *Staphylococcus aureus*, a member of  
the genus *Streptococcus*, and *Streptococcus pneumoniae*.
14. A method for inhibiting a growth of bacteria comprising the steps of  
15 contacting a composition comprising bacteria with an antibacterially effective amount of an  
antagonist that inhibits an activity of Fab I, wherein said activity is selected from the group  
consisting of:  
uncompetitive inhibition by Apo-ACP versus NADH (K<sub>i</sub>(app));  
competitive inhibition by Apo-ACP versus crotonoyl CoA;  
20 induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
binding of NADH and NADPH by FabI;  
oxidation of NADH and NADPH by FabI;  
ratio of K<sub>m</sub>app for NADH as compared to NADPH;  
25 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
kinetic mechanism;  
sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
chain length; feedback regulatory mechanism of Fab I by saturated fatty  
30 acyl CoA's;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA;

- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
negative cooperativity in the binding of CCA;  
5 formation of an dimeric quaternary structure;  
formation of an tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
coA; and  
10 NADH binding to Fab I prior to or simultaneous with ACP binding.
15. The method of claim 14 wherein said bacteria is selected from the group  
consisting of: a member of the genus *Staphylococcus*, *Staphylococcus aureus*, a member of  
the genus *Streptococcus*, and *Streptococcus pneumoniae*.
16. A method for inhibiting a Fab I polypeptide comprising the steps of  
15 contacting a composition comprising bacteria with an antibacterially effective amount of an  
antagonist that inhibits an activity of Fab I, wherein said activity is selected from the group  
consisting of:
- uncompetitive inhibition by Apo-ACP versus NADH (Ki(app));  
competitive inhibition by Apo-ACP versus crotonoyl CoA;  
20 induction of negative cooperativity with respect to CCA binding;  
use of NADH and NADPH as substrates by Fab I;  
binding of NADH and NADPH by FabI;  
oxidation of NADH and NADPH by FabI;  
ratio of Kmapp for NADH as compared to NADPH;  
25 use of NADH and crotonoyl CoA as substrates by Fab I in a sequential  
kinetic mechanism;  
sequential binding of NADH and crotonoyl CoA by Fab I;  
increasing inhibition of FabI by saturated fatty acyl CoA's of increasing  
chain length; feedback regulatory mechanism of Fab I by saturated fatty  
30 acyl CoA's;  
competitive inhibition by palmitoyl CoA versus crotonoyl CoA;

- competitive inhibition by palmitoyl CoA versus crotonoyl CoA modulation  
through binding of multiple palmitoyl CoA molecules to Fab I;  
binding of multiple palmitoyl CoA molecules to Fab I;  
negative cooperativity in the binding of CCA;  
5 formation of an dimeric quaternary structure;  
formation of an tetrameric quaternary structure;  
formation of an oligomeric quaternary structure;  
binding of Fab I by pseudo-product inhibitors beta-NADP+ or palmitoyl  
coA; and  
10 NADH binding to Fab I prior to or simultaneous with ACP binding.
17. The method of claim 16 wherein said bacteria is selected from the group  
consisting of: a member of the genus *Staphylococcus*, *Staphylococcus aureus*, a member of  
the genus *Streptococcus*, and *Streptococcus pneumoniae*.

Figure 1. The Cycle of Fatty Acid Elongation in Bacteria

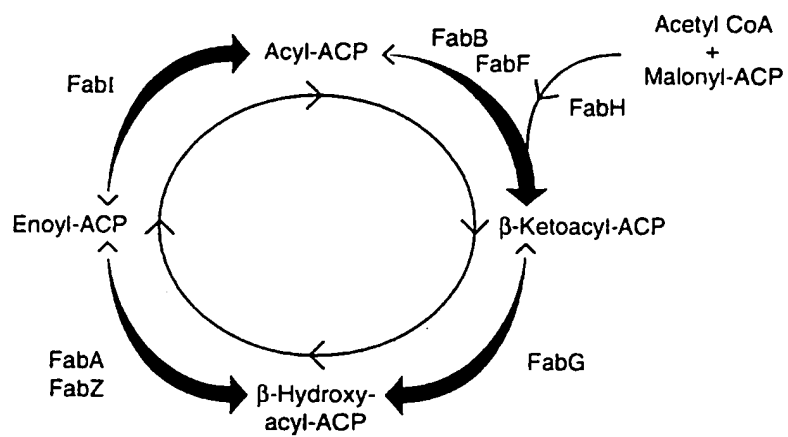
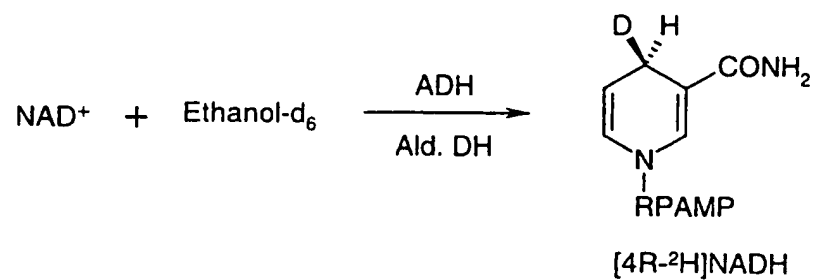


Figure 2. Synthesis of Deuterated Pyridine Nucleotides

## Synthesis of R-NADD



## Synthesis of S-NADD

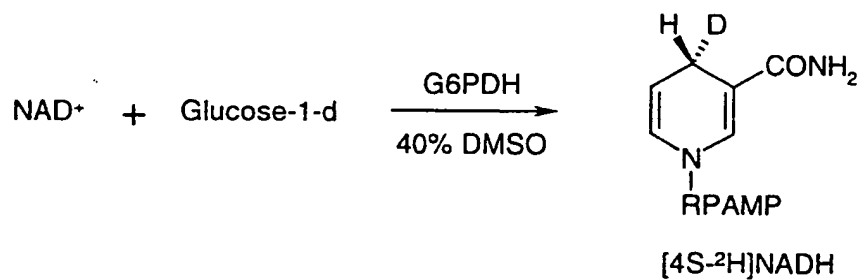


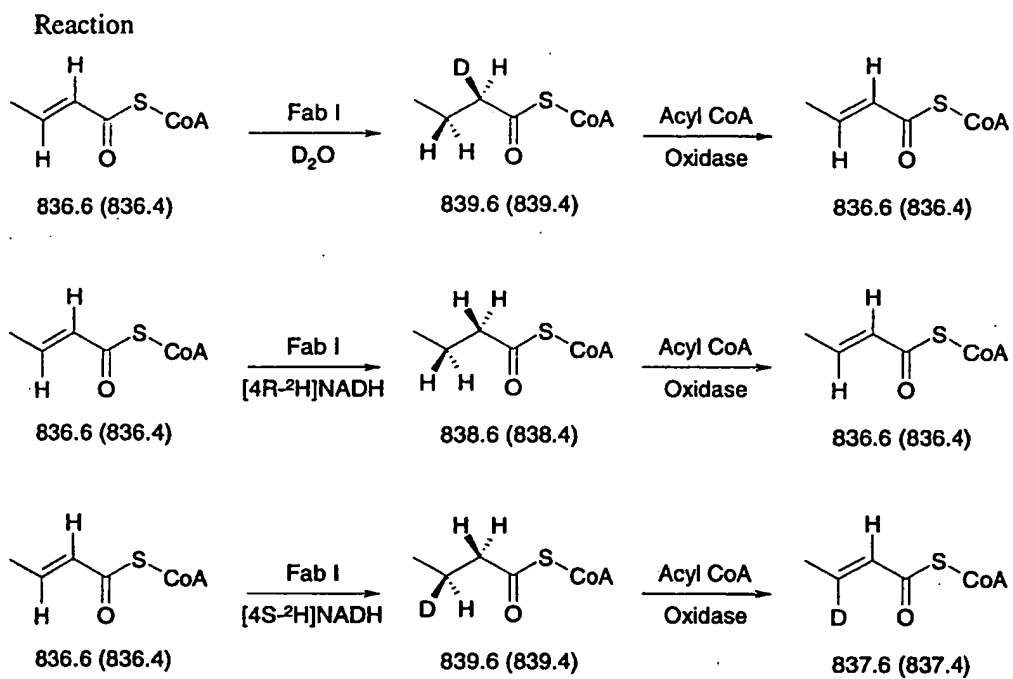
Figure 3. Predicted (Observed) Product Structures and (M+H)<sup>+</sup>'s(Based on *E. coli* Fab I)

Figure 4. Mass Spectra of Components from Reaction 3

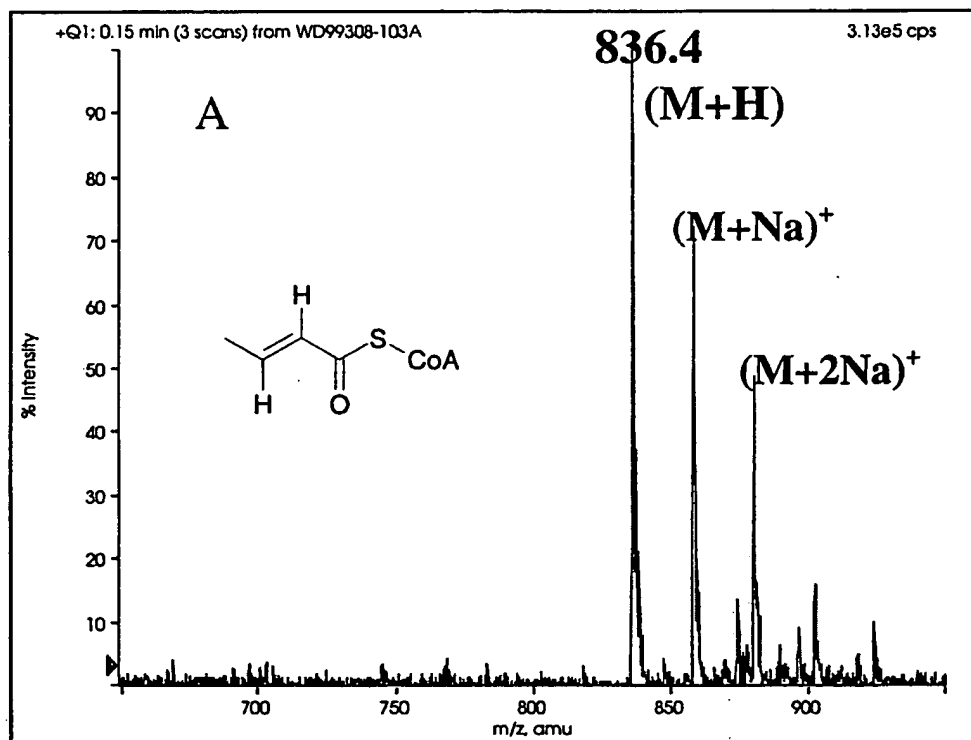




Figure 4 B

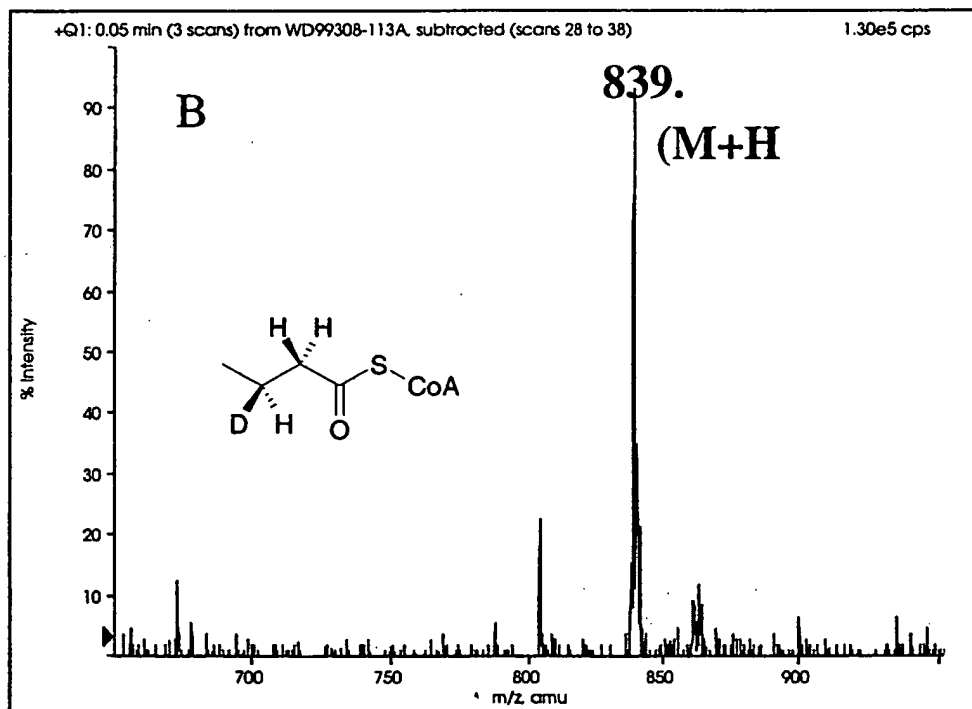


Figure 4 C

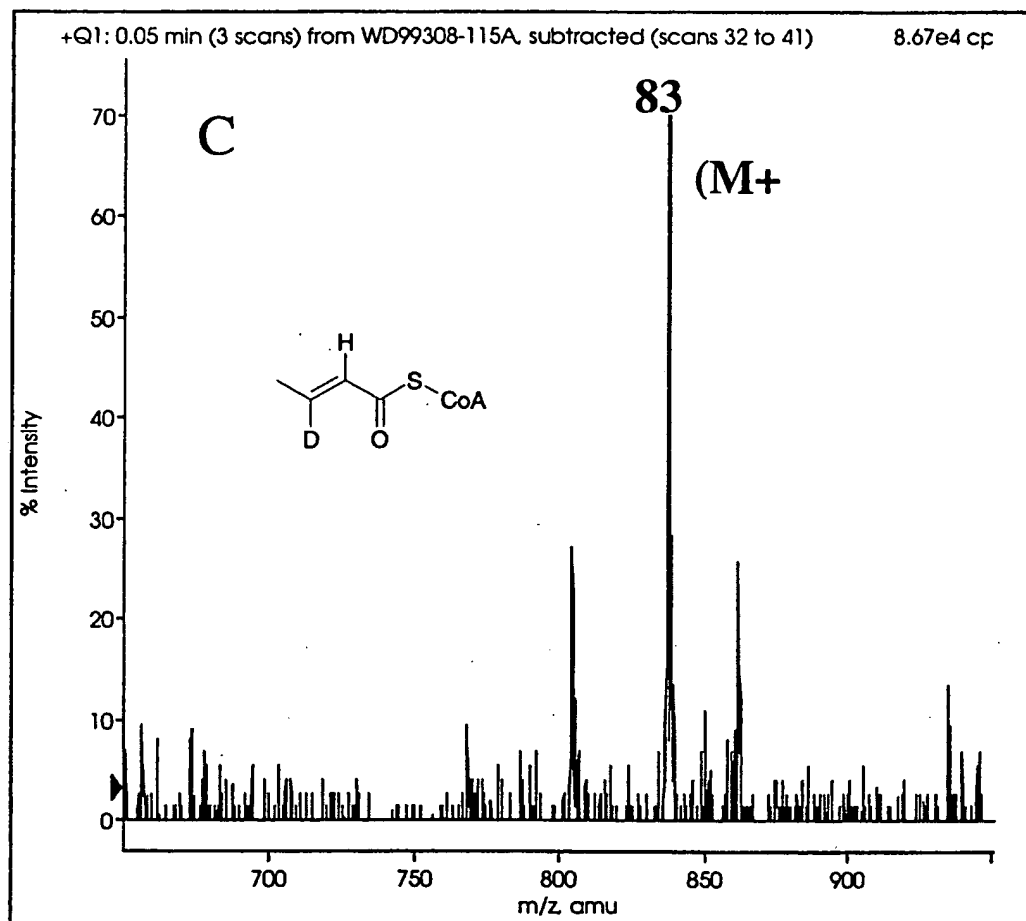


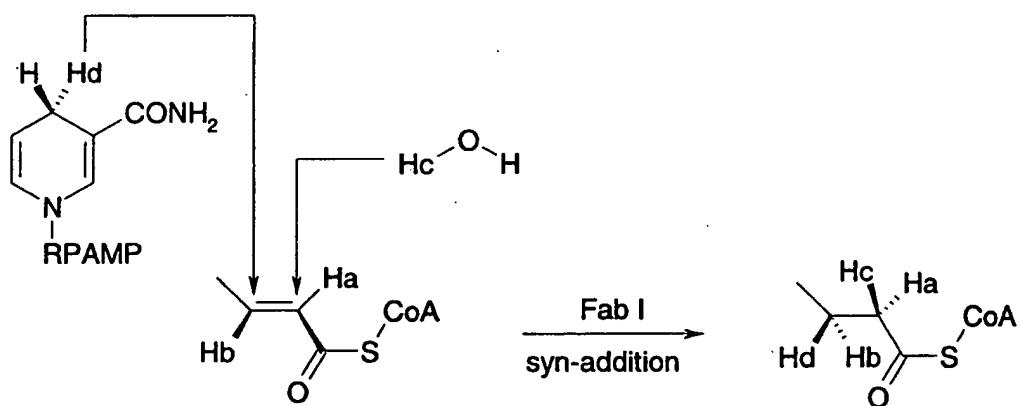
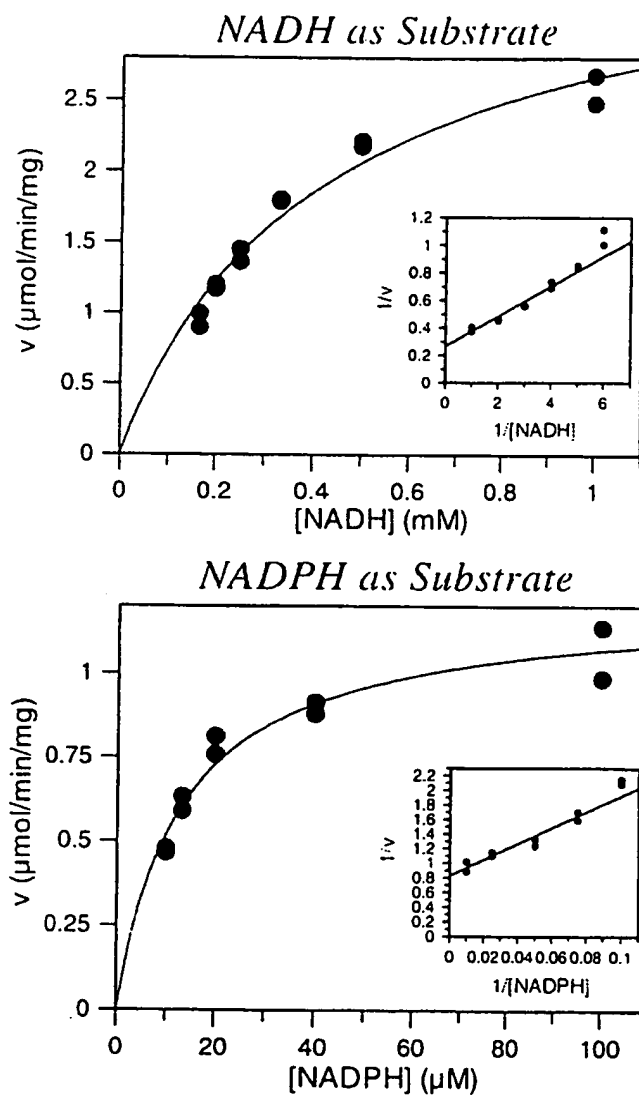
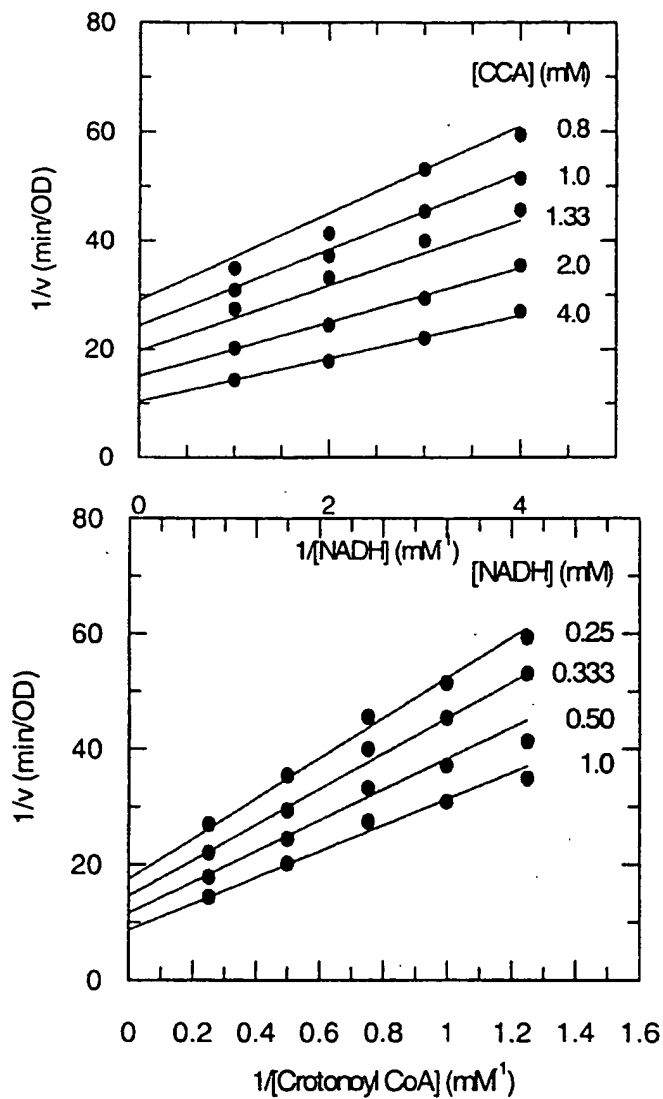
Figure 5. Stereochemical Course of *S. aureus* Fab I

Figure 6. *S. aureus* Fab I Uses Both NADPH and NADH as Substrates

	<i>NADH</i>	<i>NADPH</i>
<i>V<sub>m</sub></i> (app) (μmol/min/mg)	3.75±0.23	1.21±0.06
<i>K<sub>m</sub></i> (app) (mM)	0.41±0.06	0.013±0.002
<i>K<sub>m</sub></i> (app) CCA (mM)	3.5±0.2	1.4±0.4

Figure 7. *S. aureus* Fab I Exhibits a Sequential Mechanism

$$V_m = 0.18 \pm 0.02$$

$$K_a = 0.52 \pm 0.12 \text{ mM}$$

$$K_b = 3.3 \pm 0.6 \text{ mM}$$

$$K_{ia} = 0.22 \pm 0.06 \text{ mM}$$

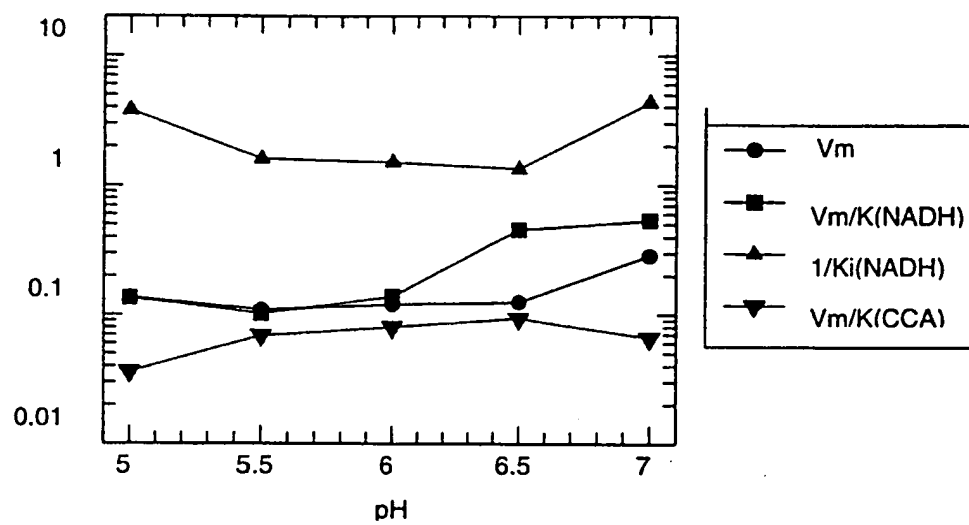
Figure 8. pH Profile of *S. aureus* Fab I

Figure 8 A Table 1. Inhibition by Saturated Fatty Acyl CoA's

<i>CoA Derivative</i>	<i>Mean IC<sub>50</sub> (μM) (n=2)</i>
<i>Acetyl</i>	>>1000
<i>n-Butyryl</i>	>>1000
<i>n-Hexanoyl</i>	576
<i>n-Octanoyl</i>	248
<i>n-Decanoyl</i>	226
<i>Lauroyl</i>	48.4
<i>Myristoyl</i>	23.1
<i>Palmitoyl</i>	10.7

Figure 9. Inhibition by Palmitoyl CoA

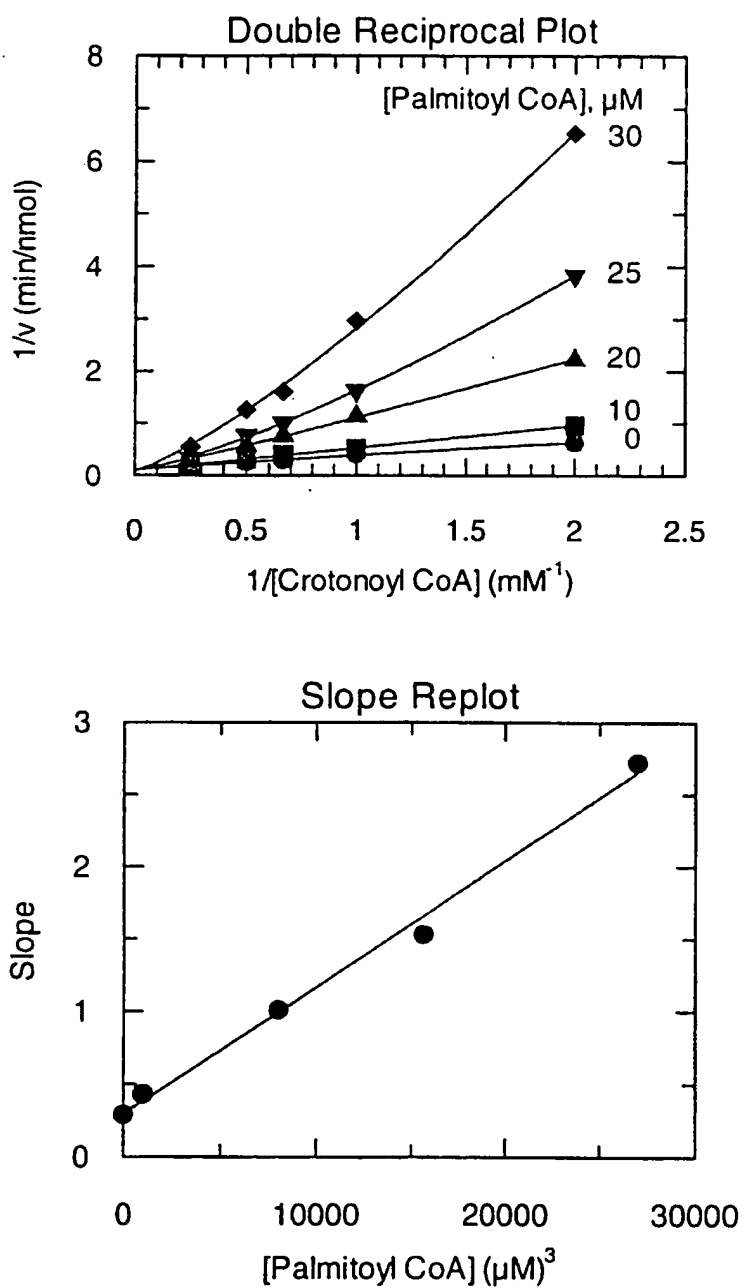




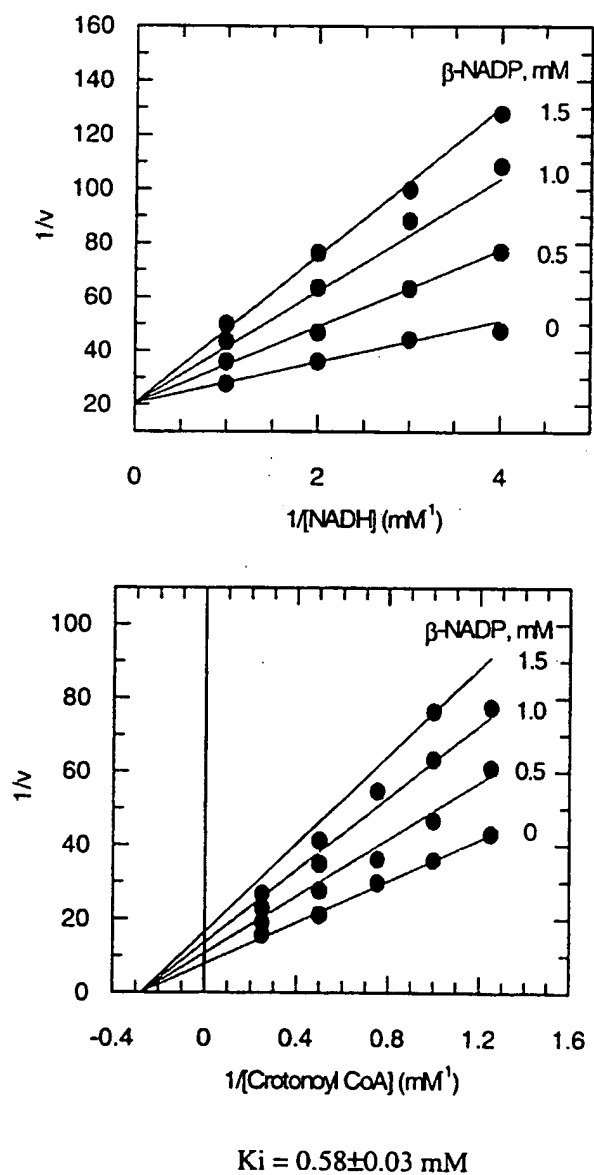
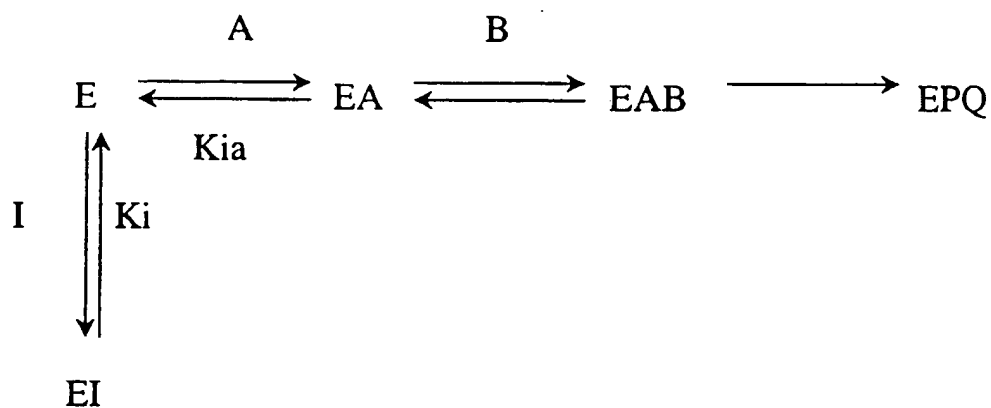
Figure 10. Inhibition by  $\beta$ -NADP<sub>+</sub>

Figure 11. Kinetic Model for Inhibition by  $\beta$ -NAPD<sup>+</sup>

$$v = \frac{V_m[A][B]}{(K_{ia}K_b + K_a[B])\left(1 + \frac{[I]}{K_i}\right) + K_b[A] + [A][B]}$$

Figure 12. Inhibition by apo-ACP vs. NADH

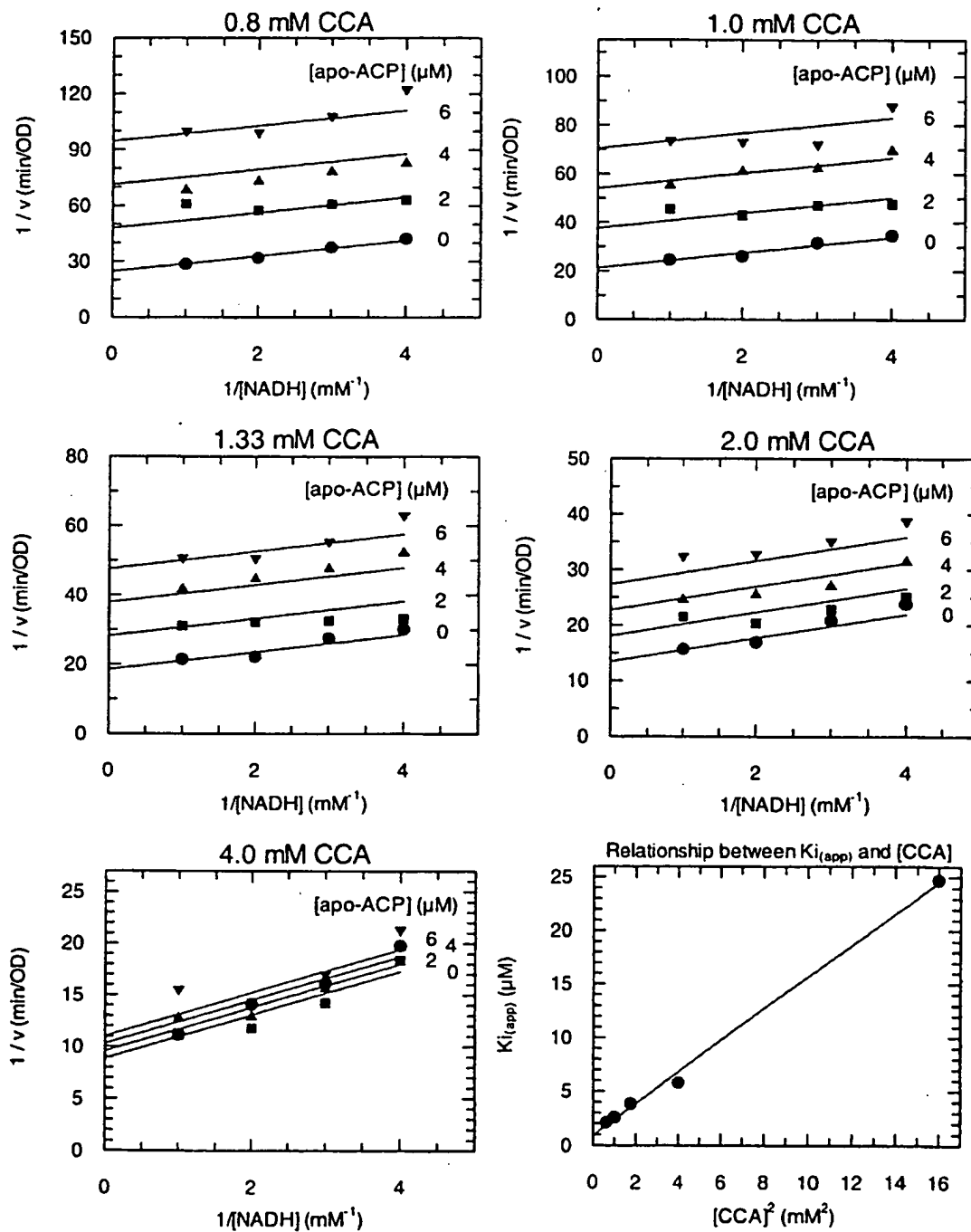


Figure 13. Inhibition by apo-ACP vs. CCA

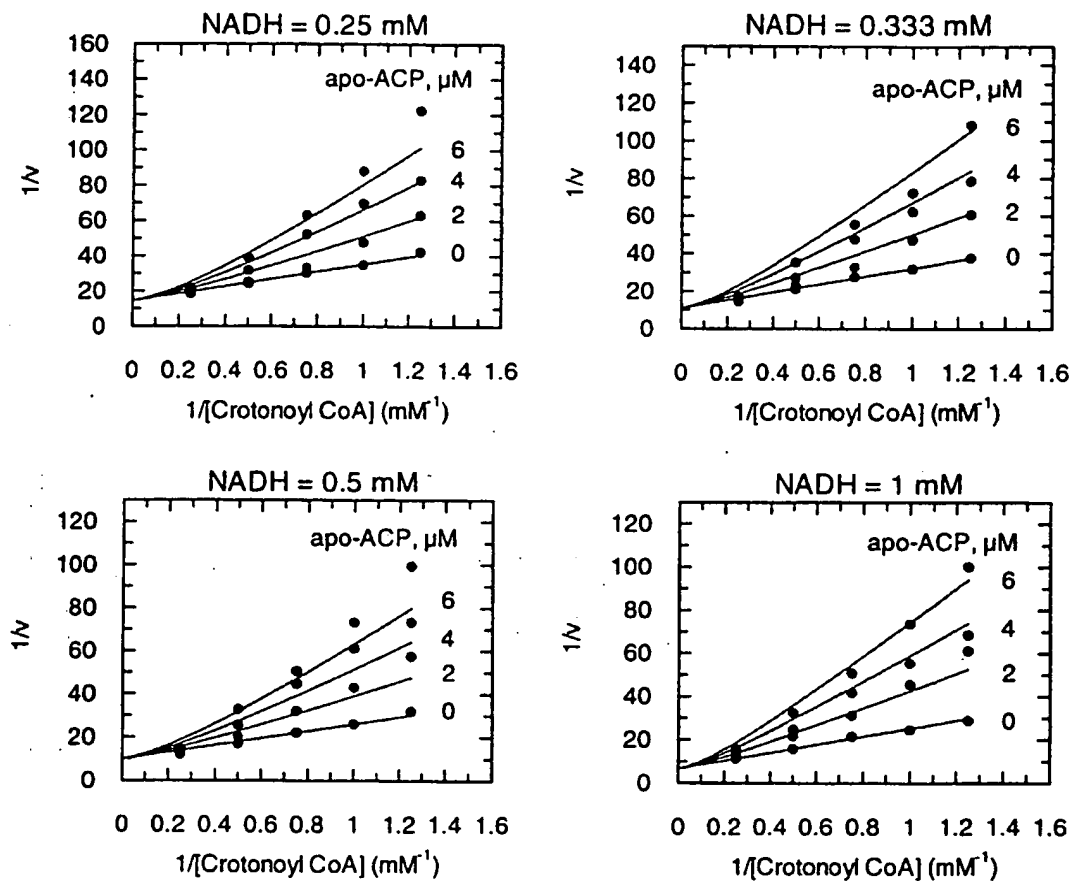
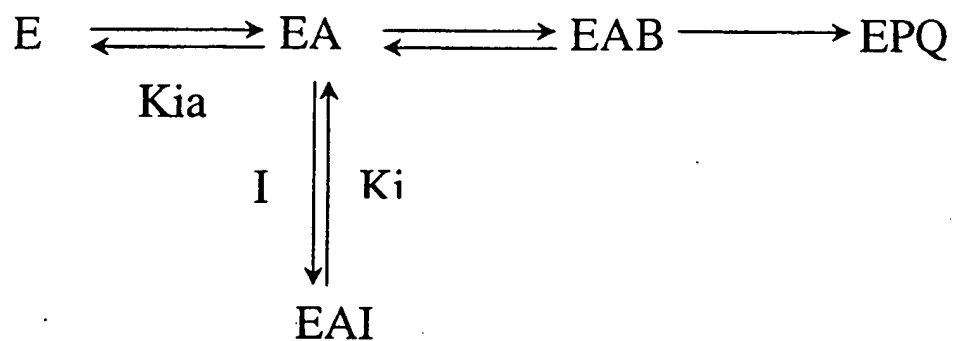


Figure 14. Minimal Kinetic Mechanism for Inhibition by apo-ACP



$$v = \frac{V_m[A][B]}{K_{ia}K_b + K_b[A]\left(1 + \frac{[I]}{K_i}\right) + K_a[B] + [A][B]}$$

Figure 15. Induced Cooperative Inhibition by apo-ACP

- Apo-ACP is uncompetitive versus NADH ( $K_i(\text{app})$  and is proportional to the square of  $[\text{CCA}]$ ).
- Apo-ACP is competitive versus crotonoyl CoA and induces negative cooperativity with respect to Cca binding.

$$v = \frac{V_m \left[ \frac{[S]}{K_S} + \frac{[S]^2}{K_S^2} + \frac{[S][I]}{\alpha K_S K_I} \right]}{\left[ 1 + \frac{2[S]}{K_S} + \frac{[S]^2}{K_S^2} + \frac{2[I]}{K_I} + \frac{[I]^2}{K_I^2} + \frac{2[S][I]}{\alpha K_S K_I} \right]}$$

$$K_i = 3 \mu\text{M}$$

$$\alpha = 15$$

## FIGURE 16

{SEQ ID NO:2}

1 MLNLENKTYV IMGIANKRSI AFGVAKVLDQ LGAKLVFTYR KERSRKELEK  
51 LLEQLNQPEA HLYQIDVQSD EEVINGFEQI GKDVGNIDGV YHSIAFANME  
101 DLRGRFSETS REGFLLAQDI SSYSLTIVAH EAKKLMPEGG SIVATTYLGG  
151 EFAVQNYNVM GVAKASLEAN VKYLALDLGP DNIRVNAISA GPRTLSAKG  
201 VGGFNTILKE IEERAPLKRN VDQVEVGKTA AYLLSDLSSG VTGENIHVDS  
251 GFHAIK

FIGURE 17

[SEQ ID NO:1]

```
1  ATGTTAAATC TTGAAAACAA AACATATGTC ATCATGGGAA TCGCTAATAA
51  GCGTAGTATT GCTTTTGGTG TCGCTAAAGT TTTAGATCAA TTAGGTGCTA
101 AATTAGTATT TACTTACCGT AAAGAACGTA GCCGTAAAGA GCTTGAAAAA
151 TTATTAGAAC AATTAAATCA ACCAGAAGCG CACTTATATC AAATTGATGT
201 TCAAAGCGAT GAAGAGGTTA TTAATGGTTT TGAGCAAATT GGTAAAGATG
251 TTGGCAATAT TGATGGTGTA TATCATTCAA TCGCATTTGC TAATATGGAA
301 GACTTACGCG GACGCTTTTC TGAAACTTCA CGTGAAGGCT TCTTGTTAGC
351 TCAAGACATT AGTTCTTACT CATTAACAAT TGTGGCTCAT GAAGCTAAAA
401 AATTAATGCC AGAAGGTGGT AGCATTGTTG CAACAACATA TTTAGGTGGC
451 GAATTCGCAG TTCAAAATTA TAATGTGATG GGTGTTGCTA AAGCGAGCTT
501 AGAAGCAAAT GTTAAATATT TAGCATTAGA CTTAGGTCCT GATAATATTC
551 GCGTTAATGC AATTTCAGCT GGTCCAATCC GTACATTAAG TGCAAAAGGT
601 GTGGGTGGTT TCAATACAAT TCTTAAAGAA ATCGAAGAGC GTGCACCTTT
651 AAAACGTAAC GTTGATCAAG TAGAAGTAGG TAAAACAGCG GCTTACTTTRT
701 TAAGTGACTT ATCAAGTGGC GTTACAGGTG AAAATATTCA TGTAGATAGC
751 GGATTCCACG CAATTAAATA A
```



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A01N 25/00, A61K 47/00
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[US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US). SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Great West Road, Middlesex TW8 9EP (GB).
- (72) Inventor; and (88) Date of publication of the international search report:  
8 February 2001
- (75) Inventor/Applicant (*for US only*): DEWOLF, Walter,

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: METHODS USING OF FAB I AND COMPOUNDS MODULATING FAB I ACTIVITY

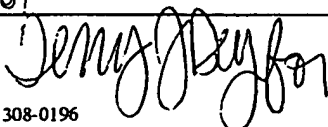
(57) Abstract: Prokaryotic FAB I polypeptides and DNA (RNA) encoding such FAB I and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such FAB I for the treatment of infection, such as bacterial infections. Antagonists against such FAB I and their use as a therapeutic to treat infections, such as staphylococcal infections are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to the presence of FAB I nucleic acid sequences and the polypeptides in a host. Also disclosed are diagnostic assays for detecting polynucleotides encoding FAB I and for detecting the polypeptide in a host.

WO 00/70017 A3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/12104

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : C12Q 1/26; A01N 25/00; A61K 47/00 US CL : 435/25, 183, 189; 514/789 According to International Patent Classification (IPC) or to both national classification and IPC														
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/25, 183, 189; 514/789  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) INDEX BIOSCIENCE, BIOSIS, CA, CAPLUS, MEDLINE, EMBASE, SCISEARCH														
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X	EP 0826774 A2 (SMITHKLINE BEECHAM CORPORATION) 04 March 1998 (03.04.98) see especially pages 20-22.	1-17												
X	JP 10-174590 A (SMITHKLINE BEECHAM CORPORATION) 30 June 1998 (06.30.98) see especially pages 40-44.	1-17												
Y	BERGLER, H. ET AL. Protein EnvM is the NADH-dependent enoyl-ACP reductase (FabI) of Escherichia coli. J. Biol. Chem., 25 February 1994, Vol. 269, No. 8, pages 5493-5496, see entire article.	2-8, 10-17												
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"><tr><td colspan="2">* Special categories of cited documents:</td></tr><tr><td>*A* document defining the general state of the art which is not considered to be of particular relevance</td><td>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr><tr><td>*E* earlier document published on or after the international filing date</td><td>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr><tr><td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td><td>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td></tr><tr><td>*O* document referring to an oral disclosure, use, exhibition or other means</td><td>*A* document member of the same patent family</td></tr><tr><td>*P* document published prior to the international filing date but later than the priority date claimed</td><td></td></tr></table>			* Special categories of cited documents:		*A* document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*E* earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*O* document referring to an oral disclosure, use, exhibition or other means	*A* document member of the same patent family	*P* document published prior to the international filing date but later than the priority date claimed	
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*O* document referring to an oral disclosure, use, exhibition or other means	*A* document member of the same patent family													
*P* document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 04 OCTOBER 2000		Date of mailing of the international search report 14 NOV 2000												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer MANJUNATH RAO  Telephone No. (703) 308-0196												

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/12104

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☒ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/12104

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1 and 9, drawn to an agonist/antagonist.

Group II, claims 2-8 and 10-17, drawn to a method of treatment using the agonist/antagonist.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Agonists/antagonists of Staphylococcal Fab I enoyl-ACP reductase and their use to treat certain conditions are well known in the art. Thus, the inventions when considered as a whole does not contribute over the prior art, see EP 0 826,774 A2 entire document.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

See claim 1 for a list of 23 species claimed.

The claims are deemed to correspond to the species listed above in the following manner:

All 23 species- claims 1-3, 5-6, 8-12, 14 and 16.

The following claims are generic: 4, 7, 13, 15 and 17.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Agonists/antagonists of Staphylococcal Fab I enoyl-ACP reductase and their use to treat certain conditions are well known in the art. Thus, the inventions when considered as a whole does not contribute over the prior art, see EP 0 826,774 A2 entire document.